

The Design and Synthesis of Conformationally Restricted and  
Epoxide-Based Peptidomimetics

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*For Michael and Max*

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## Abstract

This thesis presents research into the design and synthesis of conformationally restricted and epoxide-based peptidomimetics. The introduction reviews the general topic of peptidomimetics, agents that either imitate or block the action of a peptide at the receptor level. The development of peptidomimetics is discussed and different types of peptidomimetics are surveyed.

Chapter 1 presents the design and synthesis of conformationally restricted *cis* peptide bond mimics. The convenient preparation of dipeptide surrogates which contain a 1,2-pyrrole moiety, designed to mimic the *cis* peptide bond, is reported. Protection of the amino terminus, and chain extension, by coupling of an amino acid residue at the carboxyl terminus, are demonstrated.

Chapter 2 presents the design, synthesis, and *in vitro* testing of a series of epoxide-based peptidomimetics, designed as inhibitors of the HIV Protease (HIV PR). Included are a series of pseudosymmetrical epoxides, designed to capitalise on the unique symmetry of the HIV PR. Unfortunately, these compounds were found to be inactive. However, a further series of epoxide-based compounds, inspired by the known inhibitor of the HIV PR, 1,2-epoxy-3-(*p*-nitrophenoxy)propane, displayed good inhibitory activity.

Chapter 3 presents an investigation of the “Horner-Wadsworth-Emmons” (HWE) reaction, an extension of a synthesis presented in Chapter 2. The effect of the aldehyde reactant and of the phosphonate reagent on the stereochemical outcome of the HWE reaction is examined. The nature of the aldehyde substituent was found to influence the

stereochemistry of the product. For example, conjugated aldehydes were found to favour the formation of the *E* olefin, while alpha-branched aldehydes favoured the *Z* olefin. Further, the nature of the phosphoryl ester was shown to have a significant influence on the stereochemistry of the product. Large alkyl groups were found to favour the formation of the *E* olefin, while electron withdrawing substituents strongly favoured the formation of the *Z* olefin.

## **Introduction**

### Part 1. Development of Peptidomimetics

Numerous examples of biologically active peptides have been discovered and characterised over the past few decades.<sup>0.1</sup> Biologically active peptides act as neurotransmitters, neuromodulators, and hormones, influencing a multitude of physiological processes in a living organism.<sup>0.1, 0.2</sup> By interaction with a specific receptor, these peptides control a series of vital functions such as cell-cell communication, metabolism, immune defence, digestion, respiration, sensitivity to pain, reproduction, behaviour, and electrolyte levels.<sup>0.1</sup> Therefore, biologically active peptides are of enormous interest in medicine and medicinal chemistry.<sup>0.1</sup> Examples of such peptides, including Angiotensin II and Somatostatin, are discussed later.<sup>0.1, 0.2</sup>

The use of biologically active peptides as therapeutic agents is often severely limited by their pharmacological properties.<sup>0.1</sup> In particular, degradation in the gut and serum, poor absorption after oral ingestion, and rapid excretion through the liver and kidneys, restrict their usefulness as drugs.<sup>0.1</sup> To overcome such problems, peptidomimetics (agents that either imitate or block the action of a peptide at the receptor level) have become extremely important.<sup>0.1, 0.2</sup> A successful peptidomimetic has sufficient secondary structure and other structural features (such as functional groups) analogous to those of the original peptide to enable it to displace the peptide from its receptor. However, it must be sufficiently non-peptidic to escape the problems of degradation and bioavailability associated with peptides.<sup>0.2</sup> Further, the development of peptidomimetics with increased efficiency and selectivity has led to a reduction in side effects, and peptidomimetics

which block the action of a peptide (thereby acting as a receptor antagonist or an enzyme inhibitor) have resulted in new types of treatment for a number of diseases.<sup>0.1, 0.2</sup>

The pharmacological requirements of a peptidomimetic include metabolic stability, good bioavailability, high affinity and selectivity for its target, and minimal side effects. The usual approach to the design of peptidomimetics requires the discovery of a lead structure (a starting point for development) which can be optimised, for example, using molecular modelling programs.<sup>0.2</sup> The native peptide often serves as a lead structure, although inhibitors and structures obtained from the screening of natural products and of compound libraries are also frequently utilised.<sup>0.2</sup> Knowledge of the conformational, chemical, and electronic properties of the native peptide and its receptor, together with their mode of interaction (for example, the mechanism of enzyme catalysis), is central to the optimisation of a lead structure.<sup>0.2</sup> However, the rational design of peptidomimetics, based on the knowledge of a peptide and/or its receptor (in the absence of a lead structure), is the goal of peptidomimetics today.

## Part 2. Types of Peptidomimetics

### *Modification of a Peptide Backbone*

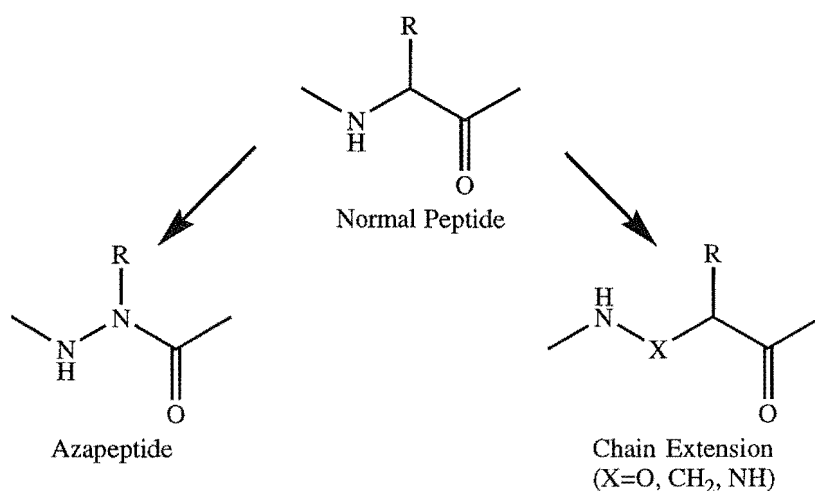
Modification of a peptide backbone refers primarily to the exchange of units in the peptide chain and/or to the introduction of additional fragments.<sup>0.2</sup> This generally leads to an increase in the biological half-life in comparison to that of the native peptide.<sup>0.1</sup> A common approach to the modification of a peptide backbone is replacement of an amide bond with a suitable mimic. This is particularly important in the development of enzyme



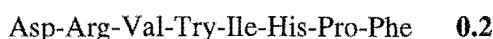
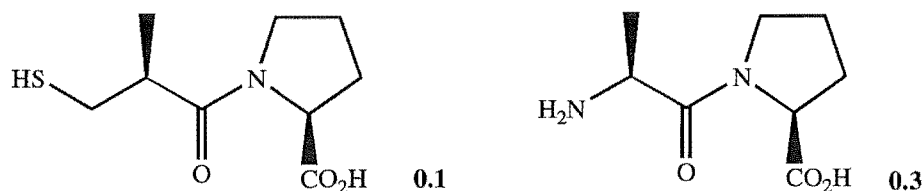
inhibitors. For example, replacement of an amide ( $\text{NH}-\text{C}=\text{O}$ ), by an alkene ( $\text{CH}=\text{CH}$ ) or a ketomethylene ( $\text{CH}_2\text{C}=\text{O}$ ), can result in a compound resistant to hydrolysis.<sup>0.2</sup> Further, the use of a hydroxyethylene group ( $\text{CH}_2\text{CHOH}$ ) in place of an amide has resulted in some potent inhibitors, for example of the Human Immunodeficiency Virus protease (HIV PR).<sup>0.3, 0.4</sup> The HIV PR is a virally encoded protease, essential to the life cycle of HIV.<sup>0.3</sup> Hydroxyethylene inhibitors of the HIV PR are discussed in the introduction to Chapter 2 of this thesis.

Further, approaches to the modification of a peptide backbone include the extension of a peptide chain by the addition of extra groups (Figure 0.1) or the exchange of individual units. For example, the carbonyl group ( $\text{C}=\text{O}$ ) of an amino acid is sometimes replaced by a thio ( $\text{C}=\text{S}$ ) or methylene ( $\text{CH}_2$ ), while the amine group ( $\text{NH}$ ) may be replaced by an oxygen or sulfur.<sup>0.2</sup> The  $\text{CH}$  group of an amino acid may also be replaced, for example, by a nitrogen atom to give an azapeptide (Figure 0.1).

Figure 0.1. Modification of the peptide backbone by the addition of extra groups or exchange of individual units.



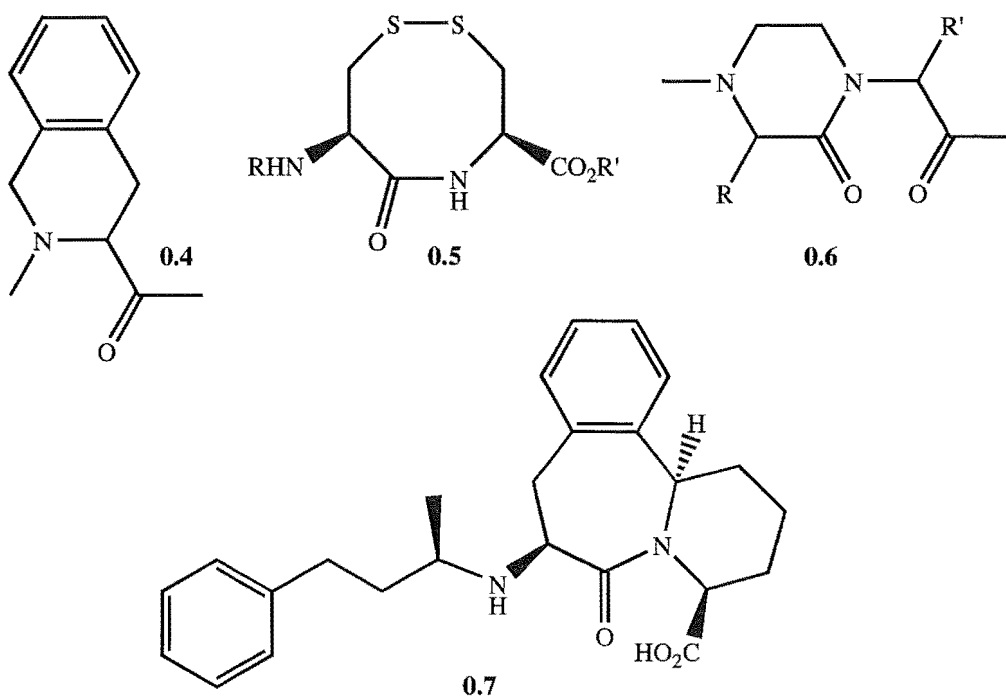
The development of the therapeutic agent Captopril (**0.1**) is an extremely important example of enzyme inhibition by modification of a peptide backbone.<sup>0.2, 0.5</sup> Captopril prevents the release of the hypertensive peptide hormone Angiotensin II (**0.2**) by inhibiting the enzyme responsible for its production—Angiotensin Converting Enzyme (ACE).<sup>0.2, 0.5</sup> Lowering of the Angiotensin II level reduces pathologically increased blood pressure and thus the incidents of heart failure and strokes.<sup>0.2, 0.5</sup> Captopril was developed from the lead structure (**0.3**), a simple dipeptide shown to be a weak inhibitor of ACE.<sup>0.2, 0.5</sup> Replacement of the terminal amine of **0.3** by a mercapto group, gave the potent and orally active drug Captopril (**0.1**).<sup>0.2, 0.5</sup> The mercapto group was introduced to co-ordinate with a zinc ion known to be located in the active site of ACE.<sup>0.2, 0.5</sup>



### *Stabilisation of Conformation by Bridging*

A successful method for the development of peptidomimetics involves the development of conformationally restricted analogues that imitate the receptor bound conformation of biologically active peptides.<sup>0.1</sup> Such analogues show increased resistance towards proteolytic degradation, enhanced metabolic stability, and better selectivity for their target.<sup>0.1</sup> The biologically active conformation of a peptide may be stabilised or constrained by the introduction of a bridge between different parts of the molecule, making the molecule more rigid.<sup>0.2</sup> A bridge may occur within a single amino acid

residue (as in **0.4**), but generally involves several residues and may either be the result of a link between two amino acid side chains (**0.5**), between two backbone units (**0.6**), or between a backbone unit and a side chain, for example, as in compound **0.7**, a potent inhibitor of ACE.<sup>0.1, 0.2</sup>



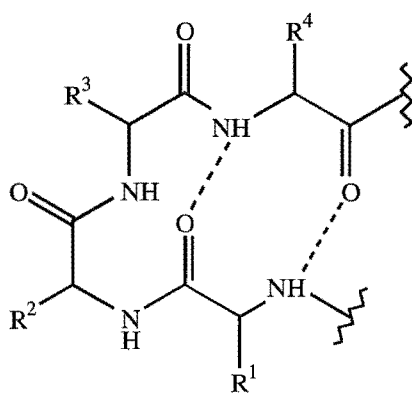
Further, conformationally constrained peptidomimetics, where the peptide (amide) bond is replaced by a ring structure, are the subject of Chapter 1 of this thesis. Examples in which the peptide bond is replaced by a tetrazole ring,<sup>0.6, 0.7</sup> a benzene ring,<sup>0.8</sup> and a pyrrole ring (in order to mimic the *cis* conformation of a peptide bond) are discussed in detail.

### *Global Restrictions of Conformation*

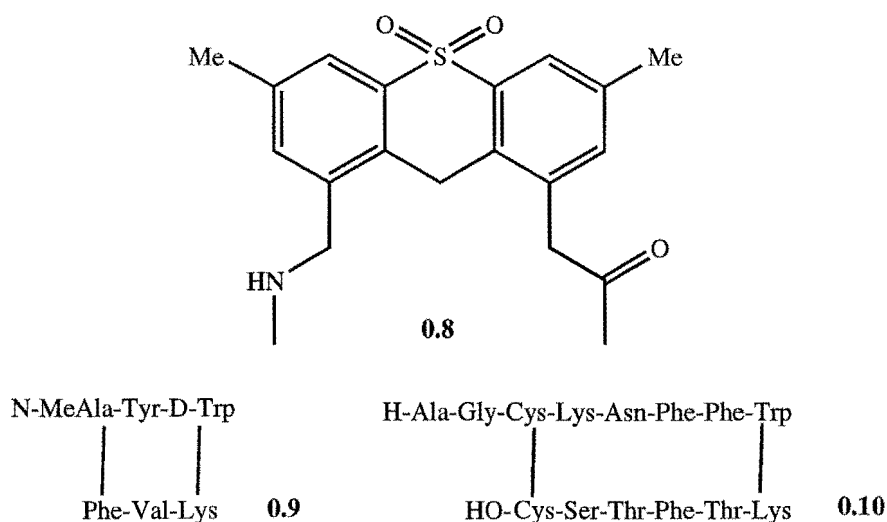
Global restrictions in the conformation of a peptide are possible by limiting the flexibility of a peptide strand through the introduction of mimetics which enforce a defined

secondary structure.<sup>0.1</sup> In addition to  $\alpha$ -helix and  $\beta$ -sheet structures, turning points are essential conformational components of peptides.<sup>0.2</sup> The  $\beta$ -turn, generally considered to be the most important turning point in a peptide chain, is formed from four amino acids and is stabilised by a hydrogen bond between the amino group of the first amino acid and the carbonyl group of the fourth amino acid, as shown in Figure 0.2.<sup>0.2</sup>

Figure 0.2. The structure of a typical  $\beta$ -turn.

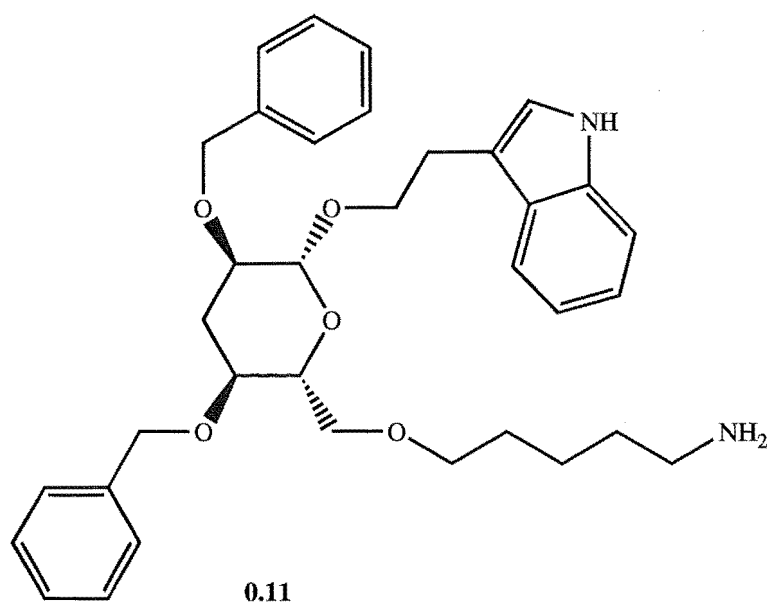


There are numerous examples of mimics designed to stabilise a  $\beta$ -turn, ranging from non-peptide components, such as **0.8**,<sup>0.2</sup> to recognisable peptide chains as in **0.9**.<sup>0.1</sup> Compound **0.9** was designed to simulate the  $\beta$ -turn, postulated between the Phe<sup>7</sup> and Thr<sup>10</sup> residues of the cyclic peptide somatostatin (**0.10**).<sup>0.1</sup> Somatostatin (**0.10**) inhibits the release of growth hormone from the pituitary gland.<sup>0.1</sup>



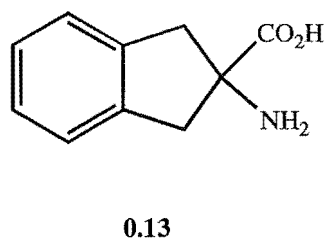
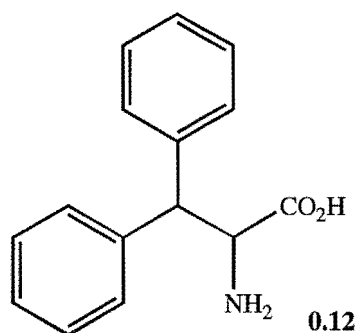
### *Scaffold Peptidomimetics*

This class of peptidomimetics utilises a completely unnatural framework to support elements of the original peptide which are responsible for its effectiveness.<sup>0.2</sup> Since it is possible to employ a framework based on a natural product or drug whose oral absorption is established, the potential exists for the development of mimetics with enhanced bioavailability. Compound **0.11** provides an example of a scaffold mimetic of somatostatin (**0.10**).<sup>0.1, 0.2</sup> Here, 3-deoxy- $\beta$ -D-glucose serves as a scaffold for the attachment of the first three side chains (Phe, Trp, and Lys) of the  $\beta$ -turn, postulated for somatostatin (**0.10**).<sup>0.1</sup> Compound **0.11** binds to the somatostatin receptor in the pituitary with an  $IC_{50}$  value of 1.3  $\mu$ M.<sup>0.1, 0.9</sup>



### *Modification of Amino Acid Side Chains*

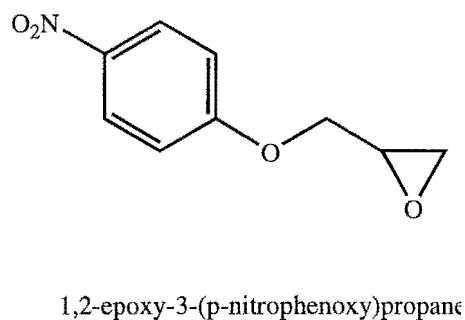
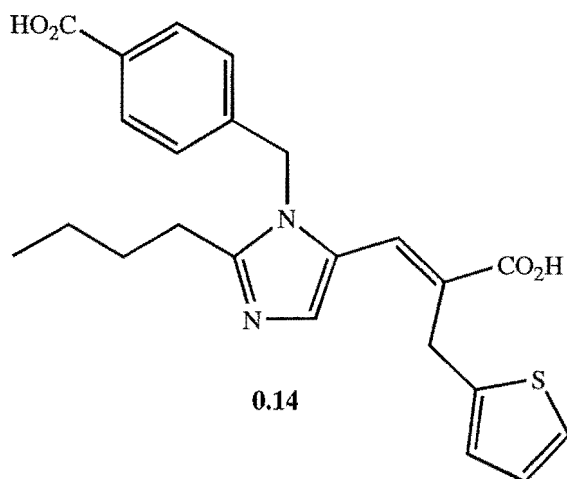
The development of conformationally restricted and/or metabolically stable peptidomimetics may be approached by the modification of amino acid side chains.<sup>0.1</sup> A common strategy involves the replacement of a natural (L) amino acid with the unnatural (D) form.<sup>0.1,0.2</sup> For example, compound **0.9**, designed to simulate the  $\beta$ -turn postulated for the cyclic peptide somatostatin (**0.10**) (discussed above), utilises D-Trp to stabilise this structural characteristic.<sup>0.1</sup> Another approach to the modification of amino acid side chains involves the introduction of sterically demanding groups which limit the range of available conformations. Compounds **0.12** and **0.13** are examples of sterically demanding analogues of the amino acid Phe and are components of peptidomimetics which bind at the Angiotensin II receptor.<sup>0.1</sup>



### Non-Peptide Mimetics

Non-peptide mimetics have generally been discovered in the course of random screening of large numbers of compounds.<sup>0.2</sup> They belong to various classes of organic compounds and usually have no apparent structural relationship with the natural peptide ligand.<sup>0.2</sup>

Compound **0.14** is an example of a non-peptide mimetic of Angiotensin II (**0.2**) which displays potent antagonistic activity at the Angiotensin II receptor.<sup>0.2</sup> A further example of a non-peptide mimetic, 1,2-epoxy-3-(*p*-nitrophenoxy)propane is an irreversible inhibitor of the HIV PR<sup>0.3, 0.10</sup> and has served as a lead structure for the development of a series of epoxide-based inhibitors of the HIV PR, described in Chapter 2 of this thesis.



## References

- 0.1. Giannis, A. Kolter, T. *Angew. Chem. Int. Ed. Engl.* **1993**, 32, 1244 and references therein.
- 0.2. Gante, J. *Angew. Chem. Int. Ed. Engl.* **1994**, 33, 1699 and references therein.
- 0.3. Meek, T. *Enzyme Inhib.* **1992**, 6, 65.
- 0.4. Wlodawer, A. Erickson, J. *Annu. Rev. Biochem.* **1993**, 62, 543.
- 0.5. Moore, G. In: *Comprehensive Medicinal Chemistry*. Editor: Hansch, C. 1st ed. Pergamon Press, Oxford. **1990**, 3, 901.
- 0.6. Yu, K. Johnson, R. *J. Org. Chem.* **1987**, 52, 2051.
- 0.7. Zabrocki, J. Dunbar, J. Marshall, K. Toth, M. Marshall, G. *J Org. Chem.* **1992**, 57, 202.
- 0.8. Elseviers, M. Van Der Auwera, L. Pepermans, H. Tourwe, D. Van Binst, G. *Biochem. Biophys. Res. Comm.* **1988**, 154 (2), 515.
- 0.9. Hirschman, R. Nicolaou, K. Pietranico, S. Leahy, E. Salvino, J. Arison, B. Cichy, M. Spoors, P. Shakespeare, W. Sprengeler, P. Hamley, P. Smith, A. Reisine, T. Raynor, K. Maechler, L. Donaldson, C. Vale, W. Freidinger, R. Cascieri, M. Strader, C. *J. Am. Chem. Soc.* **1993**, 115, 12550.
- 0.10. Rose, R. Rose, J. Salto, R. Craik, C. Stroud, R. *Biochem.* **1993**, 32, 12498.



## Chapter 1. Peptide Bonds Mimics

### Introduction

The peptide (amide) bond is one of the most important structural features of peptides and proteins.<sup>1,1</sup> Individual amino acids are connected by a peptide (amide) bond between the carboxyl group of one residue and the amino group of the subsequent residue.<sup>1,1, 1,2</sup> It is widely accepted that the peptide (amide) bond possesses considerable double bond character due to delocalisation of the lone pair of electrons on the nitrogen (Figure 1.1).<sup>1,1, 1,2</sup> Thus, amides are planar structures and a peptide bond is shorter than an N-C (alkyl) bond.<sup>1,1, 1,2</sup> Most importantly, there is a considerable barrier to bond rotation and, therefore, the peptide bond exhibits isomerism, existing in *trans* and *cis* configurations (Figure 1.2).<sup>1,1, 1,2</sup> The *trans* configuration generally predominates in secondary amides as it is considerably more stable than the *cis* configuration ( $\Delta G = -2.6$  kilocalories/mol).<sup>1,2</sup>

Figure 1.1. Delocalisation of the lone pair of electrons on the nitrogen in the peptide (amide) bond.

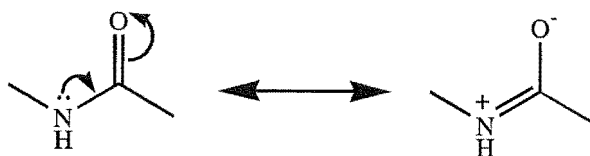
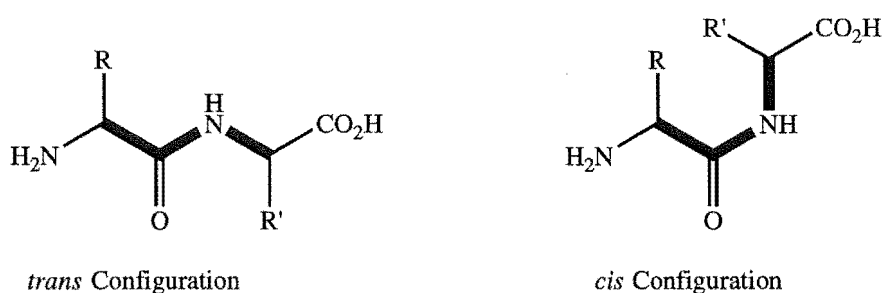


Figure 1.2. The *trans* and *cis* configurations of the peptide bond in a dipeptide.

Note: This assignment is standard practice in peptide chemistry and does not recognise the higher priority of the carbonyl.<sup>1,3, 1,4</sup>

The configuration of a peptide (amide) bond is of considerable importance in biological systems.<sup>1,1</sup> Peptides achieve their biological function through interaction with another molecule, termed the receptor. Receptor-peptide interactions are very specific, requiring the peptide to adopt a particular three dimensional structure. Thus the peptide conformation, including the *trans*: *cis* population of the peptide bond, affects biological activity. Angiotensin II, an eight residue peptide involved in blood pressure regulation (discussed in the introduction to this thesis), provides an example of this.<sup>1,3</sup> A study found that the biological activity of Angiotensin II correlates directly to the percentage of the *cis* peptide bond present between the His and Pro residues (His and Pro are located at positions six and seven of the peptide).<sup>1,3</sup> This suggests that either the *cis* peptide bond or the conformation of the peptide associated with it may be important for high affinity receptor interaction.<sup>1,3</sup>

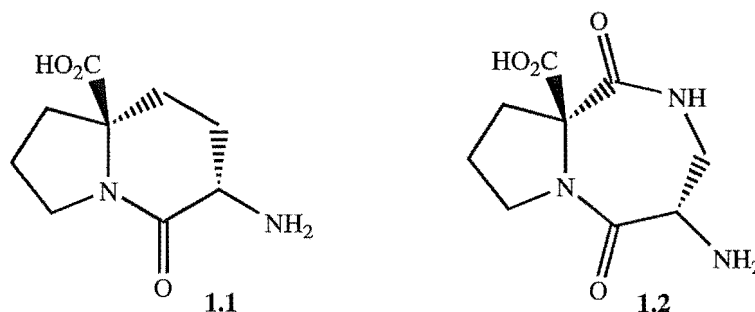
The occurrence of a *cis* peptide bond in peptides and proteins is generally associated with a Pro residue,<sup>1,2, 1,4, 1,5</sup> although there are several examples of cyclic peptides<sup>1,6, 1,7</sup> and even a membrane bound linear peptide<sup>1,8</sup> containing a *cis* peptide bond without a Pro. The barrier to bond rotation in a peptide bond involving Pro is small ( $\Delta G = -0.5$

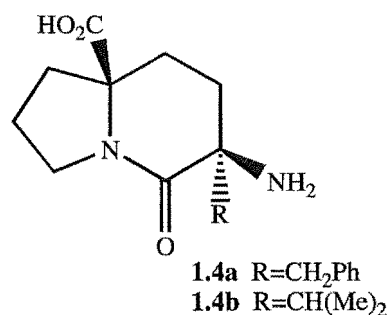
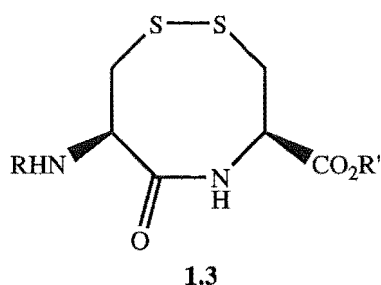
kilocalories/mol) and as a result readily detectable quantities of both *trans* and *cis* isomers are often present at equilibrium.<sup>1,2</sup> There is considerable evidence that Pro isomerism has many important biochemical roles including controlling the rate of protein folding, triggering transmembrane signalling, providing recognition in peptide antigens, and regulating the activation and breakdown of peptide hormones.<sup>1,4</sup> For example, in bovine pancreatic trypsin inhibitor, yeast cytochrome c, ribonuclease T1, and thioredoxin, the rate at which each of these proteins can fold to their native conformation is affected by site-specific mutagenesis of specific Pro residues contained in the proteins.<sup>1,9</sup> In each case, removal of critical Pro residues eliminates some folding phases detected in wild-type proteins.<sup>1,9</sup> Further, a number of peptidases, such as aminopeptidase P, do not hydrolyse *cis* peptide bonds *N*-terminal to a Pro residue.<sup>1,10</sup> Thus, the processing of peptide chains may depend on the conversion of a *cis* peptide bond to the *trans* form.<sup>1,10</sup>

The elucidation of the bioactive conformation of peptides is a challenging target. Central to this is the determination of local structural features, such as the peptide bond configuration. The use of peptidomimetics, which incorporate conformationally restricted analogues of a peptide bond into bioactive peptides, is an important approach to the study of the bioactive conformation of peptides. Further, the study of these peptide bond mimics offers the potential to discover analogues with improved stability, bioselectivity, and bioavailability. The *E* olefin is one of numerous examples of peptide bond mimics and has been successfully employed to mimic the *trans* peptide bond since it closely resembles its three dimensional structure.<sup>1,9</sup>

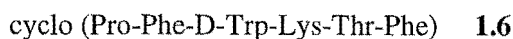
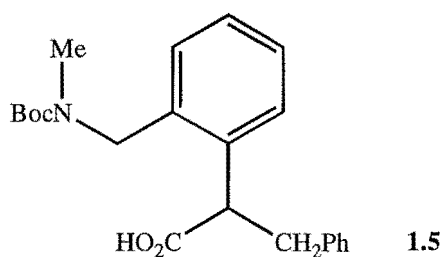
A number of conformationally restricted mimics of a *cis* peptide bond have also been developed. A frequent approach to constraining a peptide bond in the *cis* conformation is

to tether the alpha carbons of two adjacent amino acids to form cyclic dipeptide derivatives, such as **1.1**, **1.2**, and, **1.3**.<sup>1.4, 1.11</sup> Compounds of the type **1.1** have been successfully incorporated into an analogue of bradykinin<sup>1.5</sup> (discussed later) and have been used to probe the catalytic mechanism of cyclophilin.<sup>1.2</sup> Interest in cyclophilin, a family of enzymes responsible for peptide bond isomerism *N*-terminal to Pro, has increased due to the finding that they are the receptors for the immunosuppressive drug Cyclosporin A.<sup>1.2</sup> X-ray studies suggest that the active site of cyclophilin is preorganised to bind substrates in a well defined conformation characterised by a *cis* peptide bond *N*-terminal to a Pro residue.<sup>1.2</sup> Further, the crystal structures of four dipeptides bound to cyclophilin show that, in all cases, the peptide bond exists entirely in the *cis* configuration.<sup>1.2</sup> To probe the enzyme bound conformation in solution, a series of conformationally constrained *cis* dipeptide mimics of the type **1.1** were developed.<sup>1.2</sup> Two examples, **1.4a** and **1.4b** displayed exceptionally high affinity for cyclophilin, similar to that of Cyclosporin A.<sup>1.2</sup> This suggests that **1.4a** and **1.4b** closely resemble the backbone conformation of enzyme bound dipeptides and therefore that the *cis* isomer is selectively bound to cyclophilin in solution.<sup>1.2</sup>

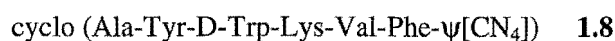
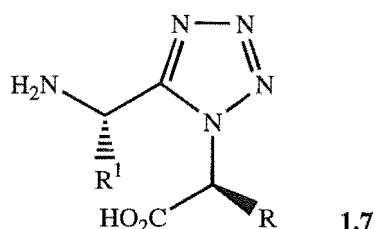




A further class of *cis* peptide bond mimics employs an aromatic ring in place of the peptide bond. By incorporating amino acids at the one and two positions of the ring, the resulting dipeptide is effectively locked in a *cis* conformation. Compound **1.5**, a Gly-Phe mimic, provides a simple racemic example.<sup>1,12</sup> Compound **1.5** was incorporated into the highly active somatostatin analogue (**1.6**) (somatostatin is a peptide which inhibits the release of growth hormone and is discussed in the introduction to this thesis).<sup>1,12</sup> The bioactive conformation of **1.6** is thought to require the *cis* configuration of the Phe-Pro peptide bond.<sup>1,12</sup> When the Phe-Pro moiety of **1.6** was replaced by the mimic (**1.5**), one of the isomers of the resultant peptidomimetic exhibited high biological activity.<sup>1,12</sup> This supports the proposed *cis* configuration for the Phe-Pro bond, in the bioactive conformation of the somatostatin analogue (**1.6**).<sup>1,12</sup>



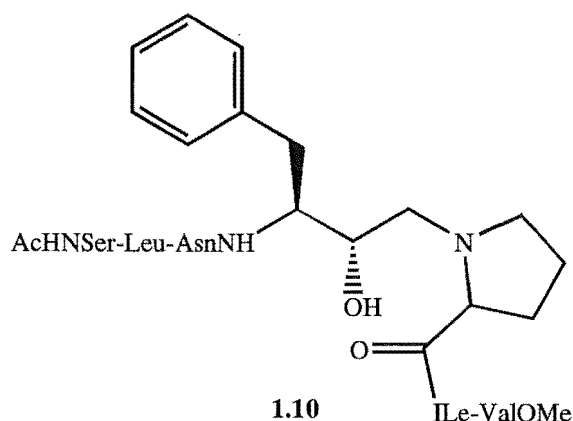
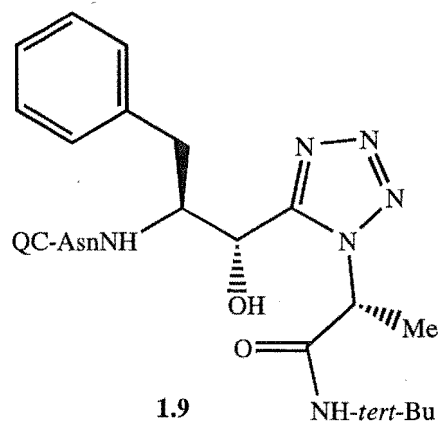
The dipeptide analogue (**1.7**), where the peptide bond is replaced by a tetrazole ring, has attracted considerable attention.<sup>1.13, 1.14, 1.15, 1.16</sup> Initial synthetic procedures to this target resulted in racemisation at one or both chiral centres adjacent to the tetrazole ring.<sup>1.13</sup> However, a recent preparation has overcome these difficulties.<sup>1.14</sup> The tetrazole dipeptide analogue (**1.7**) has been incorporated into a number of biologically active peptides, including another cyclic analogue of somatostatin giving **1.8**.<sup>1.14, 1.15, 1.17</sup> The solution structure of **1.8**, determined by NMR data and conformational analysis, was shown to be very similar to solution structures reported for cyclic hexapeptide analogues of somatostatin.<sup>1.17</sup> This demonstrates that the tetrazole ring can function as an effective *cis* peptide bond mimic in solution.<sup>1.17</sup> The potency of **1.8** was found to be 83% of that of somatostatin.<sup>1.17</sup>



( $\psi$ [CN<sub>4</sub>] represents the tetrazole ring)

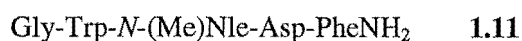
Further, tetrazole-based peptidomimetics have been employed in an attempt to elucidate the bioactive conformation of substrates and inhibitors of the Human Immunodeficiency Virus protease (HIV PR).<sup>1.18, 1.19</sup> The HIV PR is a virally encoded protease, essential to the life cycle of HIV and is discussed in detail in Chapter 2 of this thesis. The tetrazole-based peptidomimetic (**1.9**) is a analogue of the known inhibitor JG 365 (**1.10**) and was found to be a moderate inhibitor of the HIV PR (IC<sub>50</sub> 51  $\mu$ M).<sup>1.19</sup> Crystallographic evidence suggests that JG 365 (**1.10**) adopts a “*cis*-like” geometry in the active site of the

HIV PR.<sup>1.19</sup> It was reasoned that the presence of the tetrazole ring would force compound **1.9** into a bioactive conformation similar to that of JG 365 (**1.10**).<sup>1.19</sup>



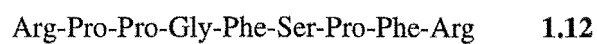
QC=*N*-(2-Quinoliny)carbonyl)

Another peptidomimetic, compound **1.11**, is an analogue of the bioactive peptide cholecystokinin, which acts as a gastrointestinal mediator and as a neurotransmitter.<sup>1.15</sup> It has been suggested that the *cis* conformation about the Trp-*N*-(Me)Nle peptide bond is responsible for the activity of **1.11** at the cholecystokinin receptor.<sup>1.15</sup> However, replacement of the Trp-*N*-(Me)Nle moiety in **1.11**, by the tetrazole mimic (Tryψ[CN<sub>4</sub>]Nle), abolished its activity.<sup>1.15</sup> This suggested that the *cis* conformation about the Trp-*N*-(Me)Nle peptide bond is not responsible for the activity of **1.11**.<sup>1.15</sup>



The biologically active peptide, bradykinin (**1.12**), is a potent mediator of blood vessel dilation, muscle contraction, pain, inflammation, and vascular permeability.<sup>1.5</sup> It has been suggested that the *cis* conformation of the Ser-Pro peptide bond is recognised at the bradykinin receptor.<sup>1.5</sup> However, peptidomimetics, in which the Ser-Pro moiety was

replaced by the *cis* peptide bond mimic (**1.7**, R/R<sup>1</sup>=Me) were inactive.<sup>1,14</sup> Further, when the *cis* peptide bond mimic (**1.1**) (mentioned above) was incorporated into **1.12** (replacing the Ser-Pro moiety) a significant decrease in activity resulted.<sup>1,5</sup> These studies suggest that the *cis* Ser-Pro conformation is not recognised at the bradykinin receptor.<sup>1,5</sup>



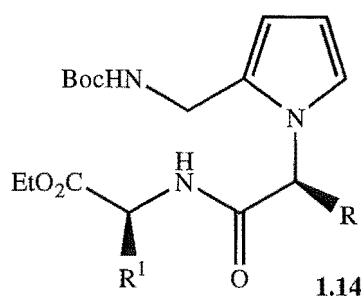
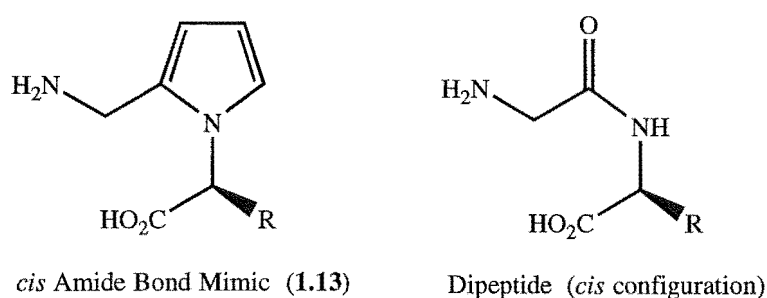


## Results and Discussion

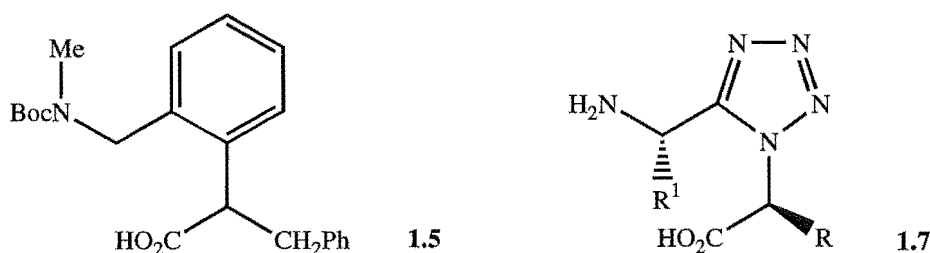
### Part 1. The Design and Synthesis of a *Cis* Peptide Bond Mimic

This section presents the design and synthesis of the heterocyclic pyrrole (**1.13**), developed as a *cis* peptide bond mimic. As discussed in the introduction to this chapter, *cis* peptide bond mimics, such as **1.7**, provide an important tool to enhance metabolic stability and probe receptor specificity in biological systems.<sup>1,16</sup> The methodology has been developed to connect a number of amino acids to Gly, giving the dipeptide analogue (**1.13**). The presence of the pyrrole ring locks **1.13** into the conformation adopted by a dipeptide possessing a *cis* peptide bond (see Figure 1.3). Further, it has been demonstrated that the amino group of **1.13** can be protected, allowing chain extension at the carboxyl terminus to give compounds of the type **1.14**.

Figure 1.3. The *cis* peptide bond mimic (**1.13**) compared to a natural dipeptide.



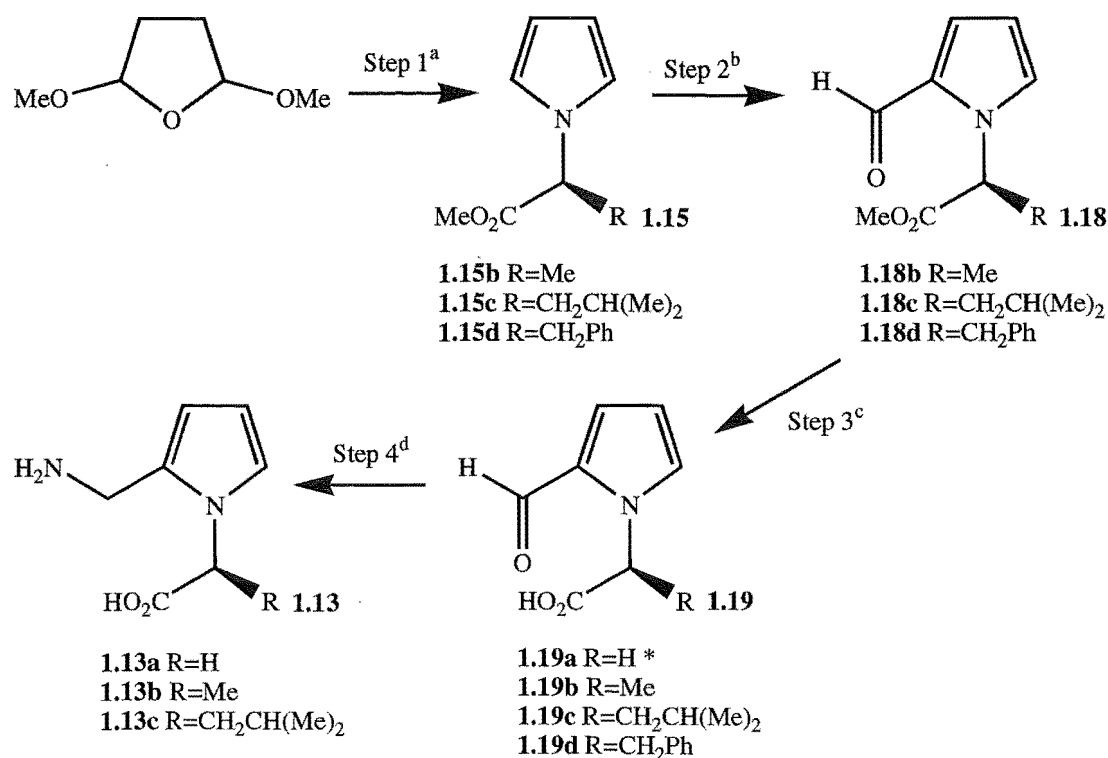
The heterocyclic pyrrole (**1.13**) possesses a number of advantages over other *cis* peptide bond mimics, such as **1.5** and **1.7**. Importantly, the synthesis of **1.13** enables control of the stereochemistry of the C terminal amino acid, whereas **1.5** is racemic at the  $\underline{\text{CHCH}_2\text{Ph}}$  carbon.<sup>1,12</sup> Further, the presence of a nitrogen in the ring of **1.13** provides a closer mimic to a natural peptide bond than the benzene ring of **1.5**, with a five membered ring (as in **1.13**) producing less steric bulk than a six membered ring (as in **1.5**). Finally, problems of racemisation and cyclisation associated with the original synthesis of the tetrazole (**1.7**) are avoided.<sup>1,16</sup>



The synthesis of compound **1.13** is shown in Scheme 1.1. Step 1, the insertion of an amino acid into 2,5-dimethoxytetrahydrofuran to give the pyrrole (**1.15**),<sup>1,20, 1.21</sup> has been demonstrated for a variety of amino acids and even proposed as a method of *N*-protection of amino acids.<sup>1,21</sup> Here, compounds **1.15b** and **1.15d** were prepared as demonstrated by Kashima *et al.*<sup>1,21</sup> and, further, **1.15c** was synthesised by this method<sup>1,21</sup> (see Scheme 1.1). Although the acidic reaction conditions encouraged polymerisation of the pyrrole nucleus, the crude reaction mixtures were successfully purified by column chromatography. To demonstrate that racemisation at the carbon alpha to the nitrogen had not occurred, **1.15b** was hydrolysed<sup>1,22</sup> to give the free acid (**1.16**). Compound **1.16** was then coupled to L-Phe ethyl ester hydrochloride, under standard conditions,<sup>1,23</sup> to give **1.17** (see Scheme 1.2). Analysis, by <sup>1</sup>H NMR spectroscopy, revealed the presence

of a single diastereoisomer in the crude reaction mixture, demonstrating the enantiomeric purity of **1.15b** (>95%).

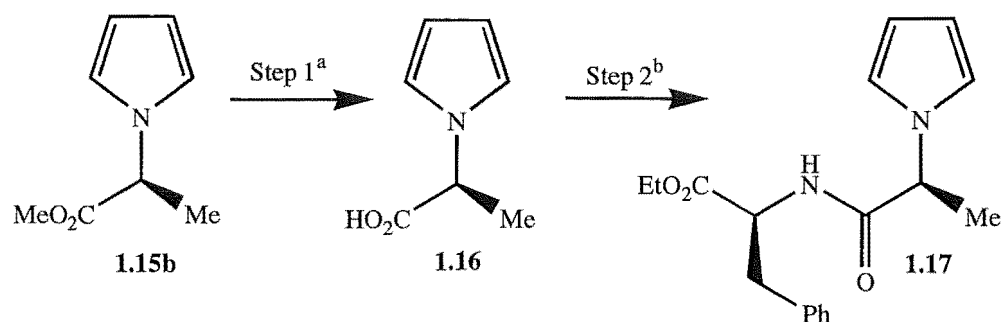
Scheme 1.1



a.<sup>1.21</sup> potassium acetate, HCl H<sub>2</sub>NCHRCO<sub>2</sub>Me, acetic acid. b.<sup>1.22</sup> trimethyl orthoformate, titanium tetrachloride, CH<sub>2</sub>Cl<sub>2</sub>. c.<sup>1.22</sup> MeOH, NaOH, water. d.<sup>1.24, 1.25</sup> ammonium acetate, sodium cyanoborohydride, MeOH.

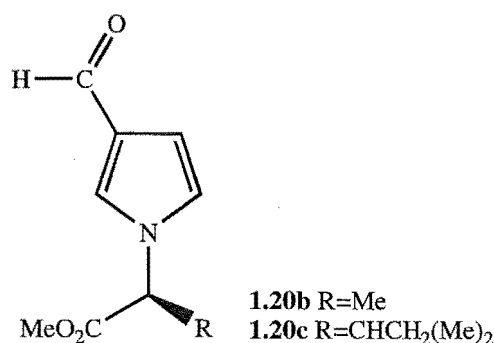
\* Compound **1.19a** was synthesised according to Nudelman *et al.*<sup>1.26</sup> (see Scheme 1.4).

Scheme 1.2

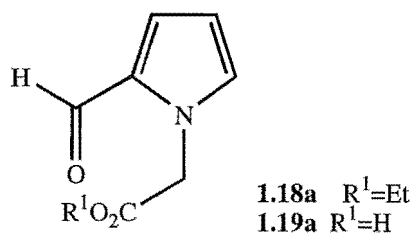


a.<sup>1.22</sup> MeOH, NaOH, water. b.<sup>1.23</sup> L-Phe ethyl ester hydrochloride, Et<sub>3</sub>N, DCC, CH<sub>2</sub>Cl<sub>2</sub>.

The formylation of **1.15** was initially achieved by the Vilsmeier formylation,<sup>1,27</sup> but the reaction gave low yields and an alternative method was investigated. Formylation of **1.15c**, using trimethylorthoformate in the presence of titanium tetrachloride (step 2, Scheme 1.1),<sup>1,22</sup> had already been demonstrated to give **1.18c** and also the 3-formylated product (**1.20c**).<sup>1,22</sup> The formylation<sup>1,22</sup> of **1.15b** and **1.15d** was investigated (step 2, Scheme 1.1). Formylation<sup>1,22</sup> of **1.15b** gave a mixture of **1.18b** (42%) and the previously unreported 3-formylated product (**1.20b**) (15%), separable by column chromatography. Interestingly, formylation<sup>1,22</sup> of **1.15d** gave only the 2-substituted product **1.18d**, also unreported.



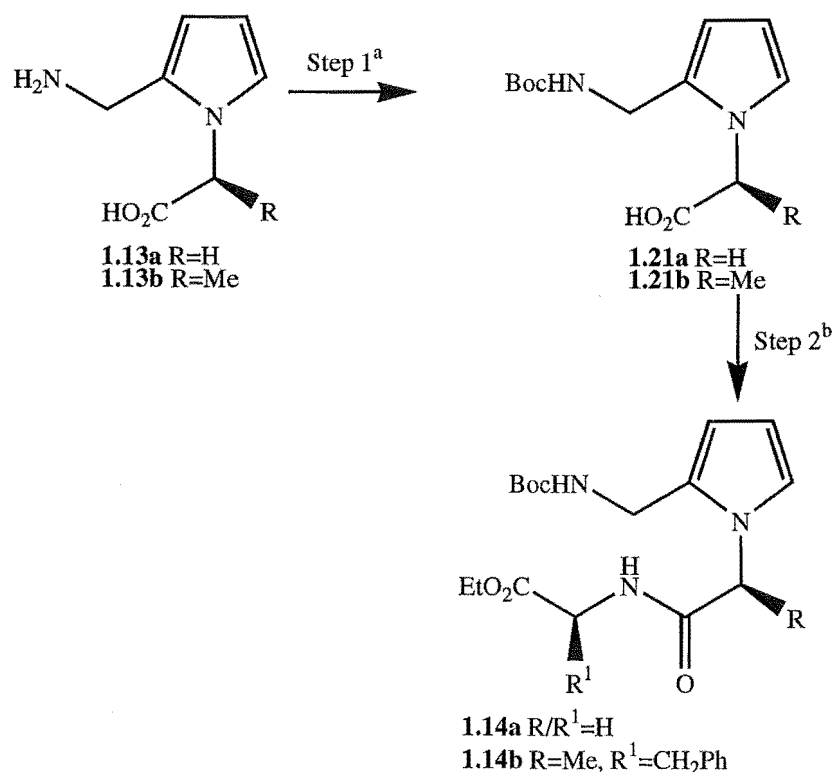
Step 3 (Scheme 1.1), the ester hydrolysis, has been demonstrated for **1.18c**, giving **1.19c**.<sup>1,22</sup> Hydrolysis of **1.18b** and **1.18d** were also achieved by this method<sup>1,22</sup> to give **1.19b** and **1.19d**, respectively (compound **1.19a** was synthesised by an literature procedure,<sup>1,26</sup> discussed later).



Step 4 (Scheme 1.1), the reductive amination<sup>1,24, 1,25</sup> of **1.19**, effectively introduces a second amino acid (Gly) at the 2-position of the pyrrole ring, giving the desired *cis*

locked dipeptide analogue (**1.13**). Initial investigations into the reductive amination<sup>1.24, 1.25</sup> of the ethyl ester (**1.18a**) (synthesised by a literature procedure<sup>1.28</sup>) gave a complex mixture of pyrrolic compounds. However, reductive amination<sup>1.24, 1.25</sup> of the free acid (**1.19a**) gave a much cleaner reaction mixture. The reaction was carried out using sodium cyanoborohydride and ammonium acetate in methanol.<sup>1.24, 1.25</sup> The product (**1.13a**) conveniently precipitated from the reaction mixture as white crystals, which proved to be water soluble and required no further purification. Similarly, reductive amination<sup>1.24, 1.25</sup> of both **1.19b** and **1.19c** gave **1.13b** and **1.13c**, respectively. Unfortunately, both these products are soluble in methanol and thus did not precipitate from the reaction mixture. Removal of the large excess of ammonium acetate was achieved by freeze drying.

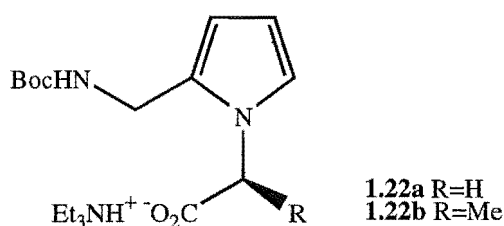
Scheme 1.3



a.<sup>1.29</sup> Et<sub>3</sub>N, "Boc-on", acetone/water. b.<sup>1.23</sup> HCl H<sub>2</sub>NCHR<sup>1</sup>CO<sub>2</sub>Et, Et<sub>3</sub>N, DCC, HOBT, CH<sub>2</sub>Cl<sub>2</sub>.

To demonstrate chain extension at the carboxyl terminus of the dipeptide mimetics and thus the feasibility of the incorporation of structures of the type **1.13** into a linear peptide, the synthetic pathway shown in Scheme 1.3 was investigated. Step 1, the protection of the amino terminus, enables chain extension of the carboxyl terminus. The reaction of both **1.13a** and **1.13b** with 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile ("Boc-on"), according to an established method,<sup>1,29</sup> gave the protected amines **1.21a** and **1.21b**, respectively.

Compounds **1.21a** and **1.21b** were initially isolated from the reaction mixture as the water soluble triethylamine salts (**1.22a** and **1.22b**). The <sup>1</sup>H NMR spectra of **1.22a** and **1.22b** revealed the presence of one equivalent of triethylamine, unable to be removed, even at 10<sup>-3</sup> mm Hg. Compounds **1.22a** and **1.22b** were converted to the corresponding free acids (**1.21a** and **1.21b**) by a standard procedure.<sup>1,29</sup>

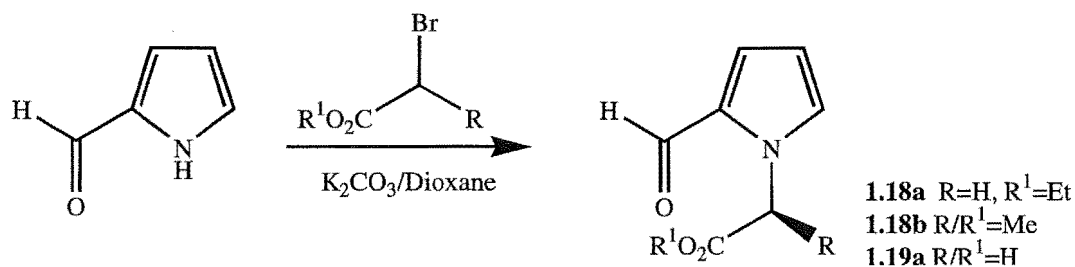


Step 2 (Scheme 1.3), the dicyclohexylcarbodiimide (DCC) coupling<sup>1,23</sup> of **1.21** and an amino acid, demonstrated chain extension at the carboxyl terminus. Initially Gly ethyl ester hydrochloride was coupled<sup>1,23</sup> with **1.21a** and the product, tentatively assigned to the structure (**1.14a**), was isolated by column chromatography. Unfortunately, **1.14a** was unstable and a mass spectrum was unable to be obtained. To investigate chain extension further, **1.21b** was coupled<sup>1,23</sup> with L-Phe ethyl ester hydrochloride, giving **1.14b** (Scheme 1.3). Compound **1.14b** was stable and able to be fully characterised. A

$^1\text{H}$  NMR of the crude reaction mixture indicated that a single stereoisomer had formed (Schemes 1.1 and 1.3).

Initially, an alternative investigation into the synthesis of compounds of the type **1.18** concentrated on the *N*-alkylation of 2-formyl pyrrole (Scheme 1.4). This reaction has been reported to give **1.18a**, **1.18b**, and **1.19a**.<sup>1,26, 1,28, 1,30</sup> Although the reaction is relatively simple and high yielding, inherent problems render it unsuitable for the development of a general method. Firstly, since a clean  $\text{S}_{\text{N}}2$  reaction cannot be guaranteed, the reaction does not allow control of the stereochemistry at the carbon alpha to the nitrogen in compounds such as **1.18b**. Secondly, there is a limited range of bromo ester starting materials commercially available, severely restricting the versatility at R. Therefore, the investigation of this synthesis was discontinued in favour of the enantioselective and general synthesis<sup>1,22</sup> shown in Scheme 1.1. However, compound **1.19a** was conveniently synthesised by this method.<sup>1,26</sup>

Scheme 1.4



## Part 2. Structure Assignment

COMPOUND **1.13a**

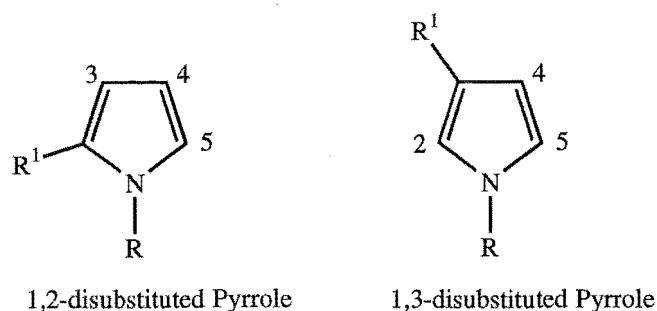
The  $^1\text{H}$  NMR spectrum of compound **1.13a** was assigned with the aid of nuclear Overhauser enhancement (nOe) experiments. Irradiation at  $\delta$  6.76 (assigned H5 on the basis of chemical shift) gave a strong enhancement at  $\delta$  6.13, thus assigned to H4 (see Figure 1.4 for numbering of the pyrrole ring). Further, an nOe was observed between the signals at  $\delta$  6.76 (H5) and  $\delta$  4.49 (thus assigned  $\text{CH}_2\text{CO}_2\text{H}$ ) and between the signals at  $\delta$  6.33 (H3) and  $\delta$  4.13 (thus assigned  $\text{CH}_2\text{NH}_2$ ). The pattern of signals for the pyrrole protons proved to be characteristic of compounds of the type **1.13** (see Table 1.1). To assign the  $^{13}\text{C}$  NMR spectrum of compound **1.13a**, a heteronuclear correlation (HETCOR) experiment was performed to distinguish the  $\text{CH}_2\text{NH}_2$  carbon at  $\delta$  41.4 from the  $\text{CH}_2\text{CO}_2\text{H}$  carbon at  $\delta$  51.6. The pattern of the pyrrole carbons also proved to be characteristic of compounds of the type **1.13** (see Table 1.2.).

Table 1.1.  $^1\text{H}$  NMR Signals ( $\delta$ ) for the Pyrrole Protons of **1.13**, **1.21**, and, **1.22**

Compound	H3	H4	H5
<b>1.13a</b>	6.33	6.13	6.76
<b>1.13b</b>	6.29	6.14	6.88
<b>1.13c</b>	6.18	5.97	6.71
<b>1.21a</b>	6.13	6.13	6.61
<b>1.21b</b>	5.99	6.14	6.77
<b>1.22a</b>	6.05	6.06	6.64
<b>1.22b</b>	5.97	6.09	6.79



Figure 1.4. Numbering of the pyrrole ring.

Table 1.2. <sup>13</sup>C NMR Signals (δ) for the Pyrrole Carbons of **1.13**, **1.21**, and **1.22**

Compound	C2	C3	C4	C5
<b>1.13a</b>	123.0	113.0	108.6	126.7
<b>1.13b</b>	122.0	112.9	108.6	123.0
<b>1.13c</b>	122.2	112.2	107.8	122.0
<b>1.21a</b>	127.4	111.5	107.9	123.3
<b>1.21b</b>	127.7	110.2	107.8	119.0
<b>1.22a</b>	128.9	109.8	107.4	124.4
<b>1.22b</b>	129.2	109.1	107.7	120.5

Amino acids, peptides, and their analogues exist in three pH inter-dependent forms: an amine salt (**A**), a zwitterion (**B**), or a carboxylate salt (**C**), as shown in Figure 1.5.<sup>1.31</sup>

Infrared and microanalytical data suggested that compound **1.13a** exists in the zwitterionic form (**B**, Figure 1.5). The infrared spectrum of **1.13a** gave absorptions at 1576 and 1399 cm<sup>-1</sup>, characteristic of the CO<sub>2</sub><sup>-</sup> anion.<sup>1.32</sup> Further, micro-analysis was consistent with the absence of a counterion, eliminating compounds both of the type **A** and **C** (Figure 1.5).

Figure 1.5. pH Inter-dependent forms of amino acids, peptides, and their analogues.

**COMPOUND 1.13b**

Compound **1.13b** was purified by recrystallisation from methanol and ethyl acetate. The  $^1\text{H}$  NMR spectrum of **1.13b** was assigned by comparison to that of **1.13a** and by homonuclear decoupling experiments. The pyrrole protons displayed a similar pattern to those of **1.13a** (see Table 1.1). Decoupling experiments showed that the signals at  $\delta$  4.65 (CHMe) and at  $\delta$  1.55 (CHMe) were mutually coupled (confirming the assignment of the signal at  $\delta$  4.65 (CHMe), which was partially obscured by the  $\text{D}_2\text{O}$  solvent peak). The  $^{13}\text{C}$  NMR spectrum of **1.13b** was assigned on the basis of a HETCOR experiment, which distinguished between signals at  $\delta$  41.4 ( $\text{CH}_2\text{NH}_2$ ) and  $\delta$  57.3 (CHMe) and enabled assignment of the pyrrole carbons (Table 1.2.). In the infrared spectrum of **1.13b**, absorptions at 1601 and  $1388\text{ cm}^{-1}$  indicated the presence of a carboxylate anion. Attempts to obtain a satisfactory micro analysis were unsuccessful.

**COMPOUND 1.13c**

The NMR spectra of **1.13c** were assigned by comparison to those of **1.13a** and **1.13b**. The signals for the pyrrole protons followed the pattern typical of compounds of the type (**1.13**) (Table 1.1). A HETCOR experiment distinguished between the signals at  $\delta$  40.4 ( $\text{CH}_2\text{NH}_2$ ) and  $\delta$  39.8 (CH $_2$ CH). An infrared spectrum gave absorptions at 1605 and  $1338\text{ cm}^{-1}$ , suggesting a carboxylate anion.

**COMPOUND 1.14a**

The  $^1\text{H}$  NMR spectrum of compound **1.14a** was assigned with the aid of nOe experiments, which distinguished between the  $\text{CH}_2\text{NHBoc}$  and  $\text{CH}_2\text{CO}$  resonances. Enhancements were obtained between the resonances at  $\delta$  4.67 ( $\text{CH}_2\text{CO}$ ) and  $\delta$  6.69 (H5) and between the resonances at  $\delta$  4.36 ( $\text{CH}_2\text{NHBoc}$ ) and  $\delta$  6.15 (H3) (see Figure 1.4 for numbering of the pyrrole ring). The  $^{13}\text{C}$  NMR spectrum of compound **1.14a** was assigned, on the basis of a HETCOR experiment which clearly distinguished the  $\text{CH}_2\text{CONH}$  carbon (at  $\delta$  50.3) from the  $\text{CH}_2\text{CO}_2\text{Et}$  carbon (at  $\delta$  41.1) and enabled the assignment of the pyrrole carbons. Unfortunately, compound **1.14a** was unstable and thus mass spectral and micro analytical data were unable to be obtained.

**COMPOUND 1.14b**

To assign the  $^1\text{H}$  NMR spectrum of compound **1.14b**, homonuclear decoupling experiments were performed to distinguish between the broad signals at  $\delta$  4.98 and  $\delta$  4.74, due to the  $\text{CHMe}$  and  $\text{CHCH}_2\text{Ph}$  protons, respectively. The  $\text{CH}_2\text{NH}$  protons gave rise to a broad doublet of doublets at  $\delta$  4.11. The  $^{13}\text{C}$  NMR spectrum compound **1.14b** was assigned by comparison to that of compound **1.21b** and the L-Phe ethyl ester cation.<sup>1.33</sup> Compound **1.14b** proved to be more stable than compound **1.14a** and a satisfactory mass spectrum was obtained.

COMPOUND **1.17**

To our knowledge, compound **1.17** is previously unreported and was therefore fully characterised. The  $^1\text{H}$  NMR spectrum of **1.17** was assigned by comparison to that of **1.15b** and L-Phe.<sup>1.21, 1.34</sup> To assign the  $^{13}\text{C}$  NMR spectrum, a HETCOR experiment was required to distinguish the resonances at  $\delta$  58.5 and  $\delta$  61.5, as the  $\text{CHMe}$  and  $\text{CHCH}_2\text{Ph}$  carbons, respectively.

COMPOUND **1.18d**

The  $^1\text{H}$  NMR spectra of 2-formylated pyrroles, such as **1.18d**, displayed a distinct pattern for signals in the pyrrole region of the spectrum. As shown in Table 1.3, resonances were found for H3 close to  $\delta$  7.0, for H4 between  $\delta$  6.2 and  $\delta$  6.4, and H5 close to  $\delta$  7.2 (see Figure 1.4 for numbering of the pyrrole ring). Further, the signal due to the aldehyde proton was found close to  $\delta$  9.5, and the alpha proton ( $\text{CHR}$ ) showed an downfield shift to lie between  $\delta$  5.9 and  $\delta$  6.1 (due to the deshielding effect of the carbonyl group). The  $^1\text{H}$  NMR spectrum of compound **1.18d** displayed this characteristic pattern of 2-formylation, although the signal for H5 was shifted upfield to  $\delta$  7.01 (Table 1.3). The aldehyde signal was also in an upfield position at  $\delta$  9.43. The signal for the alpha proton ( $\text{CHR}$ ) demonstrated a characteristic downfield shift to  $\delta$  6.06. The signal for H5 was obscured by signals due to the phenyl group, and homonuclear decoupling experiments were required to assign H5. Irradiation at  $\delta$  6.91 (H3) gave simplification at  $\delta$  6.06 (H4) and  $\delta$  7.01 (thus assigned to H5). Irradiation at  $\delta$  7.01 gave simplification at  $\delta$  6.06 (H4) and  $\delta$  6.91 (H3).

Table 1.3.  $^1\text{H}$  NMR Signals ( $\delta$ ) for 2-Formyl Pyrroles (**1.18**) & 3-Formyl Pyrroles (**1.20**)

Compound	H2	H3	H4	H5	CHO	CHR
<b>1.18b</b>	-	7.0	6.32	7.18(br)	9.52	5.90
<b>1.18c</b>	-	6.97	6.33	7.20(br)	9.53	6.10
<b>1.18d</b>	-	6.91	6.22	7.01	9.43	6.06
<b>1.20b</b>	7.41	-	6.67	6.76	9.77	4.80
<b>1.20c</b>	7.37	-	6.56	6.73	9.80	4.65

COMPOUND **1.19d**

Compound **1.19d** is previously unreported and was thus fully characterised. NMR spectra were assigned by comparison to those of compound **1.18d**.

COMPOUND **1.20b**

The  $^1\text{H}$  NMR spectrum of compound **1.20b** was assigned by comparison to literature data for compound **1.20c**.<sup>1,22</sup> The signals for the pyrrole protons exhibited a characteristic pattern, confirming 3-substitution (see Table 1.3). Further, the aldehyde proton resonated at  $\delta$  9.77 and the alpha proton (CHR) at  $\delta$  4.80. Table 1.3 demonstrates that these shifts are distinct from the signals observed for 2-formylated pyrroles, such as **1.18d** discussed above.

COMPOUNDS **1.21a** and **1.21b**

The NMR spectra of compound **1.21a** were assigned by comparison to those of compound **1.22a** and further by nOe experiments, which distinguished between the  $\text{CH}_2\text{NH}$  and  $\text{CH}_2\text{CO}_2\text{H}$  proton resonances. Irradiation at  $\delta$  6.13 (H3) gave an enhancement at  $\delta$  4.27 (thus assigned  $\text{CH}_2\text{NH}$ ) and irradiation at  $\delta$  6.61 (H5) led to an enhancement at  $\delta$  4.71 (thus assigned  $\text{CH}_2\text{CO}_2\text{H}$ ) (see Figure 1.4 for numbering of the pyrrole ring). The  $^1\text{H}$  NMR spectrum of compound **1.21b** was difficult to assign due to extreme broadening of the signals for the  $\text{CHMe}$  and  $\text{CH}_2\text{NH}$  protons and nOe experiments were performed. Irradiation at  $\delta$  5.99 (H3) gave an enhancement at  $\delta$  4.55 ( $\text{CH}_2\text{NH}$ ), and irradiation at  $\delta$  4.55 gave an enhancement at  $\delta$  5.99 (H3) and  $\delta$  4.05. Further, irradiation at  $\delta$  4.05 gave an enhancement at  $\delta$  4.55. This nOe between  $\delta$  4.05 and  $\delta$  4.55 suggested that both these signals were due to the  $\text{CH}_2\text{NH}$  signal. This was confirmed by proton-proton correlation spectroscopy (COSY), which distinctly showed correlation between these signals.

COMPOUNDS **1.22a** and **1.22b**

A marked upfield shift was observed for the signal due to H3 in the  $^1\text{H}$  NMR spectra of **1.22a** and **1.22b** (in comparison to **1.13a** and **1.13b**)—see Table 1.1. This is presumably due to the effect of the Boc group. For compound **1.22b**, nOe experiments distinguished the signals at  $\delta$  6.09 and  $\delta$  5.97, due to H4 and H3 respectively. Irradiation of the doublet of doublets at  $\delta$  4.26, due to the  $\text{CH}_2\text{NH}$  protons, gave a strong enhancement at  $\delta$  5.97 (thus assigned to H3), and irradiation at  $\delta$  5.97 gave a weak enhancement for signals due to  $\text{CH}_2\text{NH}$  and H4, confirming the assignment. However, for compound **1.22a**, nOe

experiments failed to distinguish between the signals at  $\delta$  6.05 and  $\delta$  6.06 (H3 and H4), although the broad nature of the signal at  $\delta$  6.05 suggested assignment to H3. The signal due to H3 was broadened in compounds **1.22b**, **1.21a**, and **1.21b**. To further assign the  $^1\text{H}$  NMR spectrum of compound **1.22a**, the signals due to  $\text{CH}_2\text{CO}_2\text{H}$  and  $\text{CH}_2\text{NH}$  were distinguished by a HETCOR experiment which correlated the  $^1\text{H}$  NMR resonance at  $\delta$  4.44 to the  $^{13}\text{C}$  NMR signal at  $\delta$  50.8 (assigned to the  $\text{CH}_2\text{CO}_2\text{H}$  carbon on the basis of chemical shift and distinguished from the signal for the  $\text{CH}_2\text{NH}_2$  carbon, which is broadened and close to  $\delta$  40). Table 1.2 lists the  $^{13}\text{C}$  NMR resonances for the pyrrole carbons of **1.22a** and **1.22b**. For compound **1.22b**, the signals due C3 and C4 were distinguished on the basis of a HETCOR experiment, and those for compound **1.22a** were assigned by comparison to **1.22b**. For both compounds, the signals for C3 were broadened and the resonances for C2 demonstrated a downfield shift on addition of "Boc" (in comparison to **1.13a** and **1.13b**)—see Table 1.2.

## Experimental

### General Procedure

Melting points (m.p.) were taken on a Reichert hot-stage microscope and are uncorrected. Nuclear Magnetic Resonance (NMR) spectra were recorded on either a Varian CFT300 or a XL300 spectrometer. Mass spectra (MS) were obtained on a Kratos MS80RFA spectrometer. Infrared (IR) spectra were recorded on a Shimadzu FTIR-8201PC spectrophotometer. The amino acids (glycine ethyl ester hydrochloride, L-phenylalanine ethyl ester hydrochloride, and L-leucine methyl ester hydrochloride) were commercially available.

### Preparation<sup>1.24, 1.25</sup> of *N*-Carboxymethyl-2-aminomethylpyrrole (**1.13a**)

To a stirring solution of 882 mg (5.7 mmol) of **1.19a** (synthesised by a literature procedure<sup>1.26</sup>) and 3.624 g (47 mmol) of ammonium acetate in dry methanol (MeOH) (20 ml), was added 195 mg (3.1 mmol) of sodium cyanoborohydride and stirring was continued at room temperature (r.t.). Compound **1.13a** formed as a white precipitate overnight and was isolated by filtration (585 mg, 67%) m.p. 170-172 °C.

Spectral data for **1.13a**:

<sup>1</sup>H NMR (D<sub>2</sub>O) δ 6.76 (m, 1H, H5); 6.33 (m, 1H, H3); 6.13 (m, 1H, H4); 4.49 (s, 2H, CH<sub>2</sub>CO<sub>2</sub>); 4.13 (s, 2H, CH<sub>2</sub>NH<sub>2</sub>). <sup>13</sup>C NMR (D<sub>2</sub>O) δ 177.5 (CO<sub>2</sub>); 126.7 (C5); 123.0 (C2); 113.0 (C3); 108.6 (C4); 51.6 (CH<sub>2</sub>CO); 41.4 (CH<sub>2</sub>NH). MS (m/z) 154 (M<sup>+</sup>, 59%); 152 (M<sup>+</sup>-2H, 6%); 139 (M<sup>+</sup>-NH, 8%). Anal. Calculated for C<sub>7</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>: C 48.8, H 7.0, N 16.3. Found: C 49.1, H 7.0, N 16.5. FTIR (KBr Disc) 3191(br); 2361; 1576 cm<sup>-1</sup>.



Preparation<sup>1.24, 1.25</sup> of (*S*)-*N*-(1-Carboxyethyl)-2-aminomethylpyrrole (**1.13b**)

To a stirring solution of 615 mg (3.7 mmol) of **1.19b** and 2.274 g (29.5 mmol) of ammonium acetate in MeOH (9.3 ml), was added 126 mg (2.0 mmol) of sodium cyanoborohydride. The resultant solution was stirred at r.t. for two days, and the solvent was evaporated under reduced pressure. Water (100 ml) was then added to the residue, the mixture was washed with ethyl acetate (3x), and the aqueous layer was freeze dried to remove the ammonium acetate. The resultant solid was recrystallised from methanol and ethyl acetate to give **1.13b** as white crystals (306 mg, 49%.) m.p.170 °C (decomposed).

Spectral data for **1.13b**:

<sup>1</sup>H NMR (D<sub>2</sub>O) δ 6.88 (m, 1H, H5); 6.29 (m, 1H, H3); 6.14 (m, 1H, H4); 4.65 (q, 1H, J=7.2 Hz, CHMe); 4.14 (q, 2H, J=7.3 Hz, CH<sub>2</sub>); 1.55 (d, 3H, J=7.0 Hz, CHMe).

<sup>13</sup>C NMR (D<sub>2</sub>O) δ 179.4 (CO<sub>2</sub>); 123.0 (C5); 122.0 (C2); 112.9 (C3); 108.6 (C2); 57.3 (CHMe); 41.4 (CH<sub>2</sub>NH); 18.0 (CHMe). HRMS Calculated for C<sub>8</sub>H<sub>11</sub>N<sub>2</sub>O<sub>2</sub>Na<sub>2</sub>: 213.06165. Found: 213.06167. FTIR (thin film) 3424; 2988; 1601 cm<sup>-1</sup>.

Preparation<sup>1.24, 1.25</sup> of (*S*)-*N*-(1-Carboxy-3-methylbutyl)-2-aminomethylpyrrole (**1.13c**)

To a stirring solution of 767 mg (3.5 mmol) of **1.19c** (synthesised by a literature procedure<sup>1.22</sup>) and 2.120 g (27.5 mmol) of ammonium acetate in MeOH (9.2 ml), was added 113 mg (1.8 mmol) sodium cyanoborohydride. The resultant solution was stirred at r.t. for two days and the solvent was evaporated under reduced pressure. Water (100 ml) was then added to the residue, the mixture was washed with ethyl acetate (3x), and the aqueous layer was freeze dried to remove the ammonium acetate to give a white solid (466 mg, 64%) m.p. 195 °C (decomposed).

Spectral data for **1.13c**:

$^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  6.71 (m, 1H, H5); 6.18 (m, 1H, H3); 5.97 (m, 1H, H4); 4.60 (m, 1H,  $\text{CHCO}_2$ ); 4.07 (q, 2H,  $J=13.7$  Hz,  $\text{CH}_2\text{NH}_2$ ); 1.80 (m, 2H,  $\text{CHCH}_2$ ); 1.16 (m, 1H,  $\text{CH}(\text{Me})_2$ ); 0.71 (d, 3H,  $J=6.8$  Hz, Me); 0.69 (d, 3H,  $J=6.8$  Hz, Me).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  178.7 ( $\text{CO}_2$ ); 122.2 (C2); 122.0 (C5); 112.2 (C3); 107.8 (C4); 60.1 ( $\text{CHCO}_2$ ); 40.4 ( $\text{CH}_2\text{NH}$ ); 39.8 ( $\text{CH}_2\text{CH}$ ); 24.4 ( $\text{CH}(\text{Me})_2$ ); 22.2 (Me); 21.0 (Me). HRMS Calculated for  $\text{C}_{11}\text{H}_{17}\text{N}_2\text{O}_2$ : 209.12899. Found: 209.12869. FTIR (thin film) 3453; 2955; 1677;  $1605\text{ cm}^{-1}$ .

Preparation<sup>1,23</sup> of *N*-(Ethoxycarbonylmethyl)carbamoylmethyl]-2-*tert*-butoxycarbonylaminomethylpyrrole (**1.14a**)

To a stirring solution of 70 mg (0.28 mmol) of **1.21a** in dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) (3.0 ml), were added 0.28 mmol of each glycine ethyl ester hydrochloride (39 mg), triethylamine ( $\text{Et}_3\text{N}$ ) (28 mg), dicyclohexylcarbodiimide (DCC) (58 mg), and 1-hydroxybenzotriazole (HOBT) (38 mg). The resultant solution was stirred at r.t. overnight, after which the solvent was removed under reduced pressure. Ethyl acetate was then added to the residue to precipitate the dicyclohexylurea (DCU) byproduct, which was removed by filtration. The filtrate was washed (3x) with saturated sodium bicarbonate ( $\text{NaHCO}_3$ ), dried with magnesium sulfate ( $\text{MgSO}_4$ ), and the solvent was evaporated under reduced pressure. The crude mixture was purified by column chromatography on silica (70% dichloromethane, 30% ethyl acetate) to give **1.14a** as a yellow oil (28 mg, 30%).

Spectral data for **1.14a**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  6.69 (m, 1H, H5); 6.21 (m, 1H, H4); 6.15 (m(br), 1H, H3); 4.67 (s, 2H,  $\text{CH}_2\text{CONH}$ ); 4.36 (s, 2H,  $\text{CH}_2\text{NHBoc}$ ); 4.16 (q, 2H,  $J = 7.0$  Hz,  $\text{CH}_2\text{Me}$ ); 3.95 (d, 2H,  $J = 5.5$  Hz,  $\text{CH}_2\text{CO}_2\text{Et}$ ); 1.47 (s, 9H,  $\text{C}(\text{Me})_3$ ); 1.24 (t, 3H,  $J = 7.0$  Hz,  $\text{CH}_2\text{Me}$ ).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  169.3, 168.8 (CO); 154.7 ( $\text{CO}_2\text{C}(\text{Me})_3$ ); 128.0 (C2); 123.2 (C5); 111.7 (C3); 109.1 (C2); 81.5 ( $\text{C}(\text{Me})_3$ ); 61.5 ( $\text{CH}_2\text{Me}$ ); 50.3 ( $\text{CH}_2\text{CONH}$ ); 41.1 ( $\text{CH}_2\text{CO}_2\text{Et}$ ); 39.6(br) ( $\text{CH}_2\text{NHBoc}$ ); 28.2  $\text{C}(\text{Me})_3$ ; 14.1 ( $\text{CH}_2\text{Me}$ ). FTIR (nujol) 3318(br); 2979; 2931; 1746; 1681  $\text{cm}^{-1}$ .

Preparation<sup>1,23</sup> of (S,S)-N-[(1-Ethoxycarbonyl-2-phenylethyl)-1-carbamoylethyl]-2-tert-butoxycarbonylaminomethylpyrrole (**1.14b**)

To a stirring solution of 46 mg (0.17 mmol) of **1.21b** in  $\text{CH}_2\text{Cl}_2$  (2.0 ml), were added 0.17 mmol of each L-phenylalanine ethyl ester hydrochloride (39 mg),  $\text{Et}_3\text{N}$  (16 mg), DCC (35 mg), and HOBT (24 mg). The resultant solution was stirred at r.t. overnight, after which the solvent was removed under reduced pressure. Ethyl acetate was added to the residue to precipitate the DCU byproduct, which was removed by filtration. The filtrate was washed (3x) with saturated  $\text{NaHCO}_3$ , dried with  $\text{MgSO}_4$ , and the solvent was evaporated under reduced pressure. The crude mixture was purified by column chromatography on silica (70% petroleum ether, 30% ethyl acetate) to give **1.14b** as a yellow oil (24 mg, 32%).

Spectral data for **1.14b**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.20 (m, 3H, ArH); 6.96 (m, 2H, ArH); 6.60 (m, 1H, H5); 6.17 (m, 1H, H4); 6.07 (m, 1H, H3); 4.98 (m(br), 1H,  $\text{CHMe}$ ); 4.74 (X part ABX, 1H,  $J_{\text{AX}} = 5.3$  Hz,  $J_{\text{BX}} = 6.3$  Hz,  $\text{CHCH}_2$ ); 4.11 (dd (br), 2H,  $J = 15.2, 157.3$  Hz,  $\text{CH}_2\text{NH}$ ); 4.10 (q, 2H,

$J=6.9$  Hz,  $\text{CH}_2\text{Me}$ ); 3.07 (A part ABX, 1H,  $J_{AB}=13.6$  Hz,  $J_{AX}=5.3$  Hz,  $\text{CHCH}_2$ ); 2.94 (B part ABX, 1H,  $J_{AB}=13.6$ ,  $J_{BX}=6.3$  Hz,  $\text{CHCH}_2$ ); 1.43 (s, 9H,  $\text{C}(\text{Me})_3$ ); 1.38 (d(br), 3H,  $J=7.2$  Hz,  $\text{CHMe}$ ); 1.20 (t, 3H,  $J=6.9$  Hz,  $\text{CH}_2\text{Me}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  170.9, 164.8 (CO); 154.4 ( $\text{CO}_2\text{C}(\text{Me})_3$ ); 135.6, 129.3, 128.4, 127.0 (Ar); 118.7 (C5); 111.1(br) (C4); 108.9 (C3); 106.5 (C2); 81.3 ( $\text{C}(\text{Me})_3$ ); 61.4 ( $\text{CH}_2\text{Me}$ ); 54.6 ( $\text{CHCH}_2$ ); 52.9 ( $\text{CHMe}$ ); 39.0(br) ( $\text{CH}_2\text{NH}$ ); 37.5 ( $\text{CH}_2\text{Ph}$ ); 28.2 ( $\text{C}(\text{Me})_3$ ); 17.6 ( $\text{CHMe}$ ); 14.1 ( $\text{CH}_2\text{Me}$ ). HRMS Calculated for  $\text{C}_{24}\text{H}_{33}\text{N}_3\text{O}_5$ : 443.24200. Found: 443.2419. FTIR (thin film) 2977; 1739; 1681  $\text{cm}^{-1}$ .

Preparation<sup>1.21</sup> of (*S*)-*N*-(1-Methoxycarbonyl-3-methylbutyl)pyrrole (**1.15c**)

To a stirring solution of 5.214 g (28.7 mmol) of L-leucine methyl ester and 4.610 g (47.0 mmol) of potassium acetate in acetic acid (25 ml), was added 3.670 g (28.7 mmol) of 2,5-dimethoxyterahydrofuran. The resultant solution was heated under reflux for 3.5 hours, after which water (25 ml) was added and the mixture was neutralised with solid  $\text{NaHCO}_3$ . The organic layer was extracted into ethyl acetate, dried with  $\text{MgSO}_4$ , and evaporated under reduced pressure. The crude product was purified by column chromatography on silica (70% petroleum ether, 30% ethyl acetate) to give **1.15c** as a colourless oil (2.714 g, 48%). Spectral data for **1.15c** was identical to published data.<sup>1.22</sup>

Preparation of (S,S)-N-[(1-Ethoxycarbonyl-2-phenylethyl)-1-carbamoylethyl]-pyrrole (**1.17**)

Step 1<sup>1.22</sup>

To a stirring solution of 183 mg (1.2 mmol) of **1.15b** (synthesised by a literature procedure<sup>1.21</sup>) in MeOH (10 ml), was added 60 mg (1.5 mmol) of sodium hydroxide (NaOH) in water (2.0 ml). The resultant solution was heated under reflux overnight, after which water (30 ml) was added and the mixture was washed with ether. The aqueous layer was acidified (10% hydrochloric acid (HCl)) and the product was extracted into ether, dried (MgSO<sub>4</sub>), and the solvent was evaporated under reduced pressure to give the intermediate (**1.16**) as a white solid (120 mg, 72 %) m.p. 87-89 °C.

Step 2<sup>1.23</sup>

To a stirring solution of 100 mg (0.72 mmol) of the intermediate (**1.16**) in CH<sub>2</sub>Cl<sub>2</sub> (3.0 ml), were added 0.70 mmol of each L-phenylalanine ethyl ester hydrochloride (181 mg), Et<sub>3</sub>N (80 mg), and DCC (163 mg). The resultant solution was stirred at r.t. overnight, after which the solvent was removed under reduced pressure. Ethyl acetate was then added to the residue to precipitate the DCU byproduct, which was removed by filtration. The filtrate was washed (3x) with saturated NaHCO<sub>3</sub>, dried with MgSO<sub>4</sub>, and the solvent was evaporated under reduced pressure. The crude mixture was purified by column chromatography on silica (60% dichloromethane, 30% ethyl acetate) to give (**1.17**) as a yellow oil (148 mg, 65%).

Spectral data for **1.16**:

<sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 6.75 (t, 2H, J=2.3 Hz, H<sub>2</sub>); 6.05 (t, 2H, J=2.1 Hz, H<sub>3</sub>); 4.82 (q, 1H, J=7.3 Hz, CHMe); 1.64 (d, 3H, J=7.5 Hz, CHMe).

Spectral data for **1.17**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.23 (m, 3H, ArH); 6.96 (m, 2H, ArH); 6.62 (m, 2H, H2); 6.22 (m, 2H, H3); 5.63 (d(br), 1H,  $J=7.0$  Hz, NH); 4.73 (X part ABX, 1H,  $J_{AX}=6.1$  Hz,  $J_{BX}=6.5$  Hz,  $\text{CHCH}_2$ ); 4.64 (q, 1H,  $J=7.0$  Hz,  $\text{CHMe}$ ); 4.13 (q, 1H,  $J=7.0$  Hz,  $\text{OCH}_2$ ); 3.08 (A part ABX, 1H,  $J_{AB}=7.0$  Hz,  $J_{AX}=6.1$  Hz,  $\text{CHCH}_2$ ); 2.93 (B part ABX, 1H,  $J_{AB}=7.0$  Hz,  $J_{BX}=6.5$  Hz,  $\text{CHCH}_2$ ); 1.67 (d, 3H,  $J=7.1$  Hz,  $\text{CHMe}$ ); 1.22 (t, 3H,  $J=7.0$  Hz,  $\text{CH}_2\text{Me}$ ).  
 $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  171.4, 170.9 (CO); 135.4, 129.2, 128.5, 127.1 (Ar); 119.6 (C2); 109.7 (C3); 61.5 ( $\text{CHCH}_2$ ); 58.5 ( $\text{CHMe}$ ); 52.9 ( $\text{CH}_2\text{Me}$ ); 37.5 ( $\text{CHCH}_2$ ); 17.4 ( $\text{CHMe}$ ); 14.1 ( $\text{CH}_2\text{Me}$ ). HRMS Calculated for  $\text{C}_{18}\text{H}_{22}\text{N}_2\text{O}_3$ : 314.16303 Found: 314.1631. FTIR (thin film) 3315; 2933; 2856; 1738; 1681; 1650  $\text{cm}^{-1}$ .

Preparation<sup>1.22</sup> of (*S*)-*N*-(1-Methoxycarbonylethyl)-2-formylpyrrole (**1.18b**) and (*S*)-*N*-(1-Methoxycarbonylethyl)-3-formylpyrrole (**1.20b**)

To a stirring solution of 1.004 g (9.4 mmol) of trimethylorthoformate in  $\text{CH}_2\text{Cl}_2$  (25 ml), at  $-40^\circ\text{C}$ , under a nitrogen atmosphere, was added 1.770 g (9.3 mmol) of titanium tetrachloride, followed by 1.200 g (7.8 mmol) of **1.15b** (synthesised by a literature procedure<sup>1.21</sup>), also in  $\text{CH}_2\text{Cl}_2$  (10 ml). The resultant mixture was stirred at  $-40^\circ\text{C}$  for 1.5 hours and then allowed to warm to r.t. Water (5.0 ml) was added and the organic layer was extracted into ether, dried over  $\text{MgSO}_4$ , and evaporated under reduced pressure. Column chromatography on silica (30% ethyl acetate, 70% petroleum ether) gave **1.18b** (594 mg, 42%) and **1.20b** (218 mg, 15%) as colourless oils. Spectral data for **1.18b** was identical to published data.<sup>1.30</sup>

Spectral data for **1.20b**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  9.77 (s, 1H, CHO); 7.41 (m, 1H, H<sub>2</sub>); 6.76 (m, 1H, H<sub>5</sub>); 6.67 (m, 1H, H<sub>4</sub>); 4.80 (q, 1H,  $J=7.5$  Hz,  $\text{CHMe}$ ); 3.76 (s, 3H,  $\text{CO}_2\text{Me}$ ); 1.77 (d, 3H,  $J=7.0$  Hz,  $\text{CHMe}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  185.1 (CHO); 170.5 ( $\text{CO}_2$ ); 128.2 (C<sub>2</sub>); 126.5 (C<sub>3</sub>); 122.5 (C<sub>5</sub>); 107.8 (C<sub>4</sub>); 57.1 ( $\text{CHMe}$ ); 52.6 ( $\text{OMe}$ ); 18.0 ( $\text{CHMe}$ ). HRMS Calculated for  $\text{C}_9\text{H}_{11}\text{NO}_3$ : 181.07389. Found: 181.0737. FTIR (thin film,) 3118; 2954; 2810; 1747; 1666  $\text{cm}^{-1}$ .

Preparation<sup>1,22</sup> of (*S*)-*N*-(1-Methoxycarbonyl-2-phenylethyl)-2-formylpyrrole (**1.18d**)

To a stirring solution of 1.581 g (14.9 mmol) of trimethylorthoformate in  $\text{CH}_2\text{Cl}_2$  (50 ml), at  $-40$  °C, under a nitrogen atmosphere, was added 2.827 g (14.9 mmol) of titanium tetrachloride, followed by 2.845 g (12.4 mmol) of **1.15d** (synthesised by a literature procedure<sup>1,21</sup>) also in  $\text{CH}_2\text{Cl}_2$  (2.5 ml). The resultant mixture was stirred at  $-40$  °C for 1.5 hours and then allowed to warm to r.t. Water (25 ml) was added and the organic layer was extracted into ether, dried over  $\text{MgSO}_4$ , and evaporated under reduced pressure. Column chromatography on silica (20% ethyl acetate, 80% petroleum ether) gave **1.18d** as a yellow oil (2.400 g, 75%).

Spectral data for **1.18d**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  9.43 (d, 1H,  $J=1.0$  Hz, CHO); 7.20 (m, 5H, ArH); 7.01 (m, 1H, H<sub>5</sub>); 6.91 (m, 1H, H<sub>3</sub>); 6.22 (m, 1H, H<sub>4</sub>); 6.06 (X part ABX (br), 1H,  $J_{\text{AX}}=5.9$  Hz,  $J_{\text{BX}}=9.7$  Hz,  $\text{CHCH}_2$ ); 3.74 (s, 3H, Me); 3.55 (A part ABX, 1H,  $J_{\text{AB}}=14.2$  Hz,  $J_{\text{AX}}=5.9$  Hz,  $\text{CH}_2$ ); 3.26 (B part ABX, 1H,  $J_{\text{AB}}=14.2$  Hz,  $J_{\text{BX}}=9.7$  Hz,  $\text{CH}_2$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  179.5 (CHO); 170.3 ( $\text{CO}_2$ ); 136.0, 128.9, 128.4, 126.9 (Ar); 130.6 (C<sub>5</sub>); 128.2 (C<sub>2</sub>); 125.6 (C<sub>3</sub>); 110.2 (C<sub>4</sub>); 61.0 ( $\text{CHCH}_2$ ); 52.6 (Me); 39.0 ( $\text{CH}_2$ ). MS ( $m/z$ ) 258 (22%,  $\text{M}^+$ ); 257 (100%,

M<sup>+</sup>-H); 240 (2%); 229 (2%); 228 (4%); 226 (4%); 198 (30%). FTIR (thin film) 3101; 3029; 2953; 1743; 1604 cm<sup>-1</sup>.

Preparation<sup>1,22</sup> of (S)-N-(1-Carboxyethyl)-2-formylpyrrole (**1.19b**)

To a stirring solution of 500 mg (2.7 mmol) of **1.18b** in MeOH, was added 135 mg (3.2 mmol) of NaOH in water (4.7 ml). The resultant mixture was heated under reflux overnight and then water (50 ml) was added and the mixture was washed with ether. The aqueous layer was acidified (10% HCl) and the product was extracted into ether, dried (MgSO<sub>4</sub>), and the solvent was evaporated under reduced pressure to give **1.19b** as a white solid (374 mg, 83%). Spectral data for **1.19b** was identical to published data.<sup>1,30</sup>

Preparation<sup>1,22</sup> of (S)-N-(1-Carboxy-2-phenylethyl)-2-formylpyrrole (**1.19d**)

To a stirring solution of 300 mg (1.2 mmol) of **1.18d** in MeOH (15 ml), was added 56 mg (1.4 mmol) of NaOH in water (2.0 ml). The resultant solution was heated under reflux overnight, after which water (30 ml) was added and the mixture was washed with ether. The aqueous layer was acidified (10% HCl) and the product was extracted into ether, dried (MgSO<sub>4</sub>), and the solvent was evaporated under reduced pressure to give **1.19d** as an orange solid (238 mg, 82%.) m.p. 119-121 °C.

Spectral data for **1.19d**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 9.42 (d, 1H, J=0.7 Hz, CHO); 8.15 (s(br), 1H, OH); 7.18 (3H, m, ArH); 6.96 (m, 4H, ArH, H5, and H3); 6.20 (m, 1H, H4); 6.00 (X part ABX(br), 1H, J<sub>AX</sub>=4.8 Hz, J<sub>BX</sub>=10.0 Hz, CHCH<sub>2</sub>); 3.60 (A part ABX, 1H, J<sub>AB</sub>=14.5 Hz, J<sub>AX</sub>= 4.8 Hz, CHCH<sub>2</sub>); 3.31 (B part ABX, 1H, J<sub>AB</sub>=14.5 Hz, J<sub>BX</sub>=10.0 Hz, CHCH<sub>2</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>)



$\delta$  179.9 (CHO); 174.1 (CO<sub>2</sub>); 135.9, 128.9, 128.5, 127.0 (Ar); 131.3 (C5); 126.2 (C3); 110.4 (C4); 61.2 (CHCH<sub>2</sub>); 38.4 (CHCH<sub>2</sub>). MS (m/z) 243 (67%, M<sup>+</sup>); 199 (11%, M<sup>+</sup>-CO<sub>2</sub>); 198 (5%, M<sup>+</sup>-CO<sub>2</sub>H); 170 (5%); 168 (18%); 167 (9%). FTIR (KBr Disc) 2926(br); 1737; 1658; 1622; 1530 cm<sup>-1</sup>.

Preparation<sup>1,29</sup> of *N*-(Carboxymethyl)-2-*tert*-butoxycarbonylaminomethylpyrrole (**1.21a**)

Step 1

To a stirring solution of 600 mg (3.9 mmol) of **1.13a** and 788 mg (7.8 mmol) of Et<sub>3</sub>N in acetone and water (1:1) (70 ml), was added 1921 mg (7.8 mmol) of 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile ("Boc-on"). The resultant solution was stirred at r.t overnight, filtrated, and the solvent was removed from the filtrate under reduced pressure to give the intermediate (**1.22a**) as a colourless oil (818 mg, 59%).

Step 2

A solution of 818 mg (2.3 mmol) of the intermediate (**1.22a**) in water (20 ml), at 0 °C, was adjusted to pH 2 with 5 M HCl. The product was then extracted into ethyl acetate, washed with 5% HCl, and dried (MgSO<sub>4</sub>). The solvent was removed under reduced pressure to yield **1.21a** as a colourless oil (489 mg, 49%).

Spectral data for the intermediate (**1.22a**):

<sup>1</sup>H NMR (D<sub>2</sub>O)  $\delta$  6.64 (m, 1H, H5); 6.06 (m, 1H, H4); 6.05 (m(br), 1H, H3); 4.44 (s, 2H, CH<sub>2</sub>CO); 4.23 (s(br), 2H, CH<sub>2</sub>NH); 3.12 (q, 6H, J=7.5 Hz, N(CH<sub>2</sub>Me)<sub>3</sub>); 1.20 (t, 9H, J=7.0 Hz, N(CH<sub>2</sub>Me)<sub>3</sub>); 1.40 (s, 9H, C(Me)<sub>3</sub>). <sup>13</sup>C NMR (D<sub>2</sub>O)  $\delta$  177.0 (CO<sub>2</sub><sup>-</sup>); 157.2 (CO<sub>2</sub>C(Me)<sub>3</sub>); 128.9 (C2); 124.4 (C5); 109.8(br) (C3); 107.6 (C4); 82.9 (C(Me)<sub>3</sub>); 50.8 (CH<sub>2</sub>CO); 47.4 N(CH<sub>2</sub>Me)<sub>3</sub>); 40.9(br) (CH<sub>2</sub>NH); 28.5 (C(Me)<sub>3</sub>); 9.0 (N(CH<sub>2</sub>Me)<sub>3</sub>).

Spectral data for **1.21a**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.65 (s(br), 1H, OH); 6.61 (m, 1H, H5); 6.13 (m, 2H, H4 and H3); 4.71 (s, 2H,  $\text{CH}_2\text{CO}$ ); 4.27 (s, 2H,  $\text{CH}_2\text{NH}$ ); 1.45 (s, 9H,  $\text{C}(\text{Me})_3$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  176.9 ( $\text{CO}_2\text{H}$ ); 154.9 ( $\text{CO}_2\text{C}(\text{Me})_3$ ); 127.4 (C2); 123.3 (C5); 111.5 (C3); 107.9 (C4); 81.7 ( $\text{C}(\text{Me})_3$ ); 48.1 ( $\text{CH}_2\text{CO}$ ), 39.5(br) ( $\text{CH}_2\text{NH}$ ); 28.3 ( $\text{C}(\text{Me})_3$ ). MS (m/z) 254 (15%,  $\text{M}^+$ ); 197 (30%,  $\text{M}^+-\text{Bu}$ ); 153 (17%). FTIR (nujol) 4325; 2724; 1727  $\text{cm}^{-1}$ .

Preparation<sup>1,29</sup> of (S)-N-(1-Carboxyethyl)-2-tert-butoxycarbonylaminomethyl-pyrrole (**1.21b**)

#### Step 1

To a stirring solution of 54 mg (0.32 mmol) of **1.13b** and 65 mg (0.64 mmol) of  $\text{Et}_3\text{N}$  in acetone and water (1:1) (5.5 ml), was added 158 mg (0.64 mmol) of "Boc-on". The resultant solution was stirred at r.t. overnight, filtrated, and the solvent was removed from the filtrate under reduced pressure to give the intermediate (**1.22b**) as a colourless oil (65 mg, 55%).

#### Step 2

A solution of 65 mg (0.18 mmol) of the intermediate (**1.22b**) in water (1.0 ml), at 0 °C, and was adjusted to pH 2 with 5 M HCl. The product was then extracted into ethyl acetate, washed with 5% HCl, and dried ( $\text{MgSO}_4$ ). The solvent was removed under reduced pressure to yield **1.21b** as a colourless oil (37 mg, 43%).

Spectral data for the intermediate (**1.22b**):

$^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  6.79 (m, 1H, H5); 6.09 (m, 1H, H4); 5.97 (m, 1H, H3); 4.60 (q, 1H,  $\text{J}=7.0$  Hz,  $\text{CHMe}$ ); 4.26 (dd, 2H,  $\text{J}=16.2, 58.9$  Hz,  $\text{CH}_2\text{NH}$ ); 3.10 (q, 6H,  $\text{J}=7.3$  Hz,  $\text{N}(\text{CH}_2\text{Me})_3$ ); 1.50 (d, 3H,  $\text{J}=7.3$  Hz,  $\text{CHMe}$ ); 1.41 (s, 9H,  $\text{C}(\text{Me})_3$ ); 1.19 (t, 9H,  $\text{J}=5.6$  Hz,

$\text{N}(\text{CH}_2\text{Me})_3$ .  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  179.8 ( $\text{CO}_2^-$ ); 157.3 ( $\text{CO}_2\text{C}(\text{Me})_3$ ); 129.2 (C2); 120.5 (C5); 109.1 (C3); 107.7 (C4); 83.0 ( $\text{C}(\text{Me})_3$ ); 57.0 ( $\text{CHMe}$ ); 47.3 ( $\text{N}(\text{CH}_2\text{Me})_3$ ); 41.1(br) ( $\text{CH}_2\text{NH}$ ); 28.5 ( $\text{C}(\text{Me})_3$ ); 19.3 ( $\text{CHMe}$ ); 9.1 ( $\text{N}(\text{CH}_2\text{Me})_3$ ).

Spectral data for **1.21b**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  9.08 (s(br), 1H, OH); 6.77 (m, 1H, H5); 6.14 (m, 1H, H4); 5.99 (m, 1H, H3); 4.55 (m (br), 1H,  $\text{CH}_2\text{NH}$ ); 4.29 (m(br), 1H,  $\text{CHMe}$ ); 4.05 (m (br), 1H,  $\text{CH}_2\text{NH}$ ); 1.68 (d, 3H,  $J=7.4\text{Hz}$ ,  $\text{CHMe}$ ); 1.40 (s, 9H,  $\text{C}(\text{Me})_3$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  176.9 ( $\text{CO}_2\text{H}$ ); 155.1 ( $\text{CO}_2\text{C}(\text{Me})_3$ ); 127.7 (C2); 119.0 (C5); 110.2(br) (C3); 107.8 (C4); 81.4 ( $\text{C}(\text{Me})_3$ ); 52.9 ( $\text{CHMe}$ ); 39.2(br) ( $\text{CH}_2\text{NH}$ ); 28.2 ( $\text{C}(\text{Me})_3$ ); 17.7 ( $\text{CHMe}$ ). MS (m/z) 268 (55%,  $\text{M}^+$ ); 224 (5%,  $\text{M}^+ - \text{CO}_2$ ); 224 (8%,  $\text{M}^+ - \text{CO}_2\text{H}$ ); 212 (66%); 211 (92%,  $\text{M}^+ - \text{Bu}$ , 92%). FTIR (nujol) 3363; 2977; 1733; 1682  $\text{cm}^{-1}$ .

## References

- 1.1. Schnur, D. Yuh, Y. Dalton, D. *J. Org. Chem.* **1989**, *54*, 3779.
- 1.2. Germanas, J. Kim, K. Dumas, J. In: *Recent Advances in Peptidomimetics*. Editor: Abell, A. JAI Press, Connecticut. **1997**, in press.
- 1.3. Liakopoulou-Kyriakides, M. Galaray, R. *Biochem.* **1979**, *18* (10), 1952.
- 1.4. Curran, T. McEnaney, P. *Tetrahedron Lett.* **1995**, *36* (2), 191.
- 1.5. Gramberg, D. Robinson, J. *Tetrahedron Lett.* **1994**, *35* (6), 861.
- 1.6. Mierke, D. Yamazaki, T. Said-Nejad, O. Felder, E. Goodman, M. *J. Am. Chem. Soc.* **1989**, *111*, 6847.
- 1.7. Kessler, H. Anders, U. Schudok, M. *J. Am. Chem. Soc.* **1990**, *112*, 5908.
- 1.8. Bairaktari, E. Mierke, D. Mammi, S. Peggion, E. *J. Am. Chem. Soc.* **1990**, *112*, 5383.
- 1.9. Texter, F. Spencer, D. Rosenstein, R. Matthews, R. *Biochem.* **1992**, *31* (5), 5687.
- 1.10. Yaron, A. Naider, F. *Crit. Revs. Biochem. Mol Biol.* **1993**, *28* (1), 31.
- 1.11. Cumberbatch, S. North, M. Zagotto, G. *J. Chem. Soc. Chem. Comm.* **1993**, 642.
- 1.12. Elseviers, M. Van Der Auwera, L. Pepermans, H. Tourwe, D. Van Binst, G. *Biochem. Biophys. Res. Comm.* **1988**, *154*, (2), 515.
- 1.13. Yu, K. Johnson, R. *J. Org. Chem.* **1987**, *52*, 2051.
- 1.14. Zabrocki, J. Dunbar, J. Marshall, K. Toth, M. Marshall, G. *J Org. Chem.* **1992**, *57*, 202.
- 1.15. Boteju, L. Zalewska, T. Yamamura, H. Hruby, V. *Bioorg. Med. Chem. Lett.* **1993**, *3* (10), 2011.
- 1.16. Zabrocki, J. Smith, D. Dunbar, J. Iijima, H. Marshall, G. *J. Am. Chem. Soc.* **1988**, *110*, 5875.

- 1.17. Brensen, D. Zabrocki, J. Slomczynska, U. Head, R. Kao, J. Marshall, G.  
*Biopolymers*. **1995**, 36 (2), 181.
- 1.18. Garofalo, A. Tarnus, C. Remy, J. Leppik, R. Piriou, F. Harris, B. Pelton, J. In:  
*Peptides: Chemistry, Structure and Biology*. Editors: Rivier, J. Marshall, G. ESCOM,  
Leiden, **1990**, 833.
- 1.19. Abell, A. Foulds, G. *J. Chem Soc. Perkin Trans I*. **1997**, in press.
- 1.10. Josey, A. Jenner, E. *J. Org. Chem.* **1962**, 27, 2466.
- 1.21. Kashima, C. Maruyama, T. Harada, K. Hibi, S. Omote, Y. *J. Chem. Res.* **1988**, 62.
- 1.22. Chan, T. Lee, S. *J. Org. Chem.* **1983**, 48, 3059.
- 1.23. Windridge, G. Jorgensen, E. *J. Am. Chem. Soc.* **1971**, 93 (23), 6318.
- 1.24. Lane, C. *Synth.* **1975**, March, 135.
- 1.25. Borch, R. Bernstein, M. Dupont Durst, H. *J. Am. Chem. Soc.* **1971**, 93 (12), 2897.
- 1.26. Nudelman, A. Karoly, H. Braun, F. *J. Med. Chem.* **1978**, 21 (9), 962.
- 1.27. March, J. *Advanced Organic Chemistry*. 1st. ed. J. Wiley and Son Inc., New  
York. **1968**, 417.
- 1.28. Olsson, K. Pernemalm, P. *Acta Chem. Scand.* **1979**, 33B, 125.
- 1.29. Itoh, M. Hagiwara, D. Kamiya, T. *Tetrahedron Lett.* **1975**, 49, 4393.
- 1.30. Dickerson, J. Roberts, D. Millar, C. Lloyd, R. Rix, C. *Tob. Sci.* **1976**, 20, 71.
- 1.31. Seager, S. Slabaugh, M. *Chemistry for Today*. 1st. ed. West Publishing  
Company, St. Paul. **1987**, 576.
- 1.32. Silverstein, R. Bassler, G. Morrill, T. *Spectrometric Identification of Organic  
Compounds*. 4th. ed. John Wiley & Sons, Inc., New York. **1981**, 264.
- 1.33.. Bremser, W. Ernst, L. Fachinger, W. Gerhards, R. Hardt, A. Lewis, P.  
*Carbon-13 NMR Spectral Data*. 4th. ed. VCH Verlagsgesellschaft mbH, Weinheim,  
**1987**.

- 1.34. Pouchet, C. *The Aldrich Library of NMR Spectra*. 2nd. ed. Aldrich Chemical Company, Inc., Wisconsin. **1983**, 2, 248C.

## Chapter 2. Epoxide-Based Inhibitors of the HIV Protease

### Introduction

The spread of Acquired Immune Deficiency Syndrome (AIDS) has reached catastrophic dimensions.<sup>2.1, 2.2, 2.3</sup> It is currently estimated that there are ten million people infected and that this figure could rise to 20 million by the year 2000.<sup>2.3</sup> This pandemic has promoted an unprecedented scientific and clinical effort to understand and to combat this lethal disease.<sup>2.1, 2.2</sup>

AIDS results from infection of the lymphocytes by the Human Immunodeficiency Virus (HIV), causing serious defects in cell mediated immunity.<sup>2.1</sup> This results in opportunistic infections, neurological and neoplastic diseases, and ultimately death.<sup>2.1</sup> An understanding of the molecular events critical to the replication of HIV has enabled the development of chemotherapeutic interventions. Inhibitors of reverse transcriptase, such as azidothymidine (AZT), and more recently HIV protease inhibitors are currently in clinical use for the treatment of AIDS.<sup>2.2, 2.4</sup>

### Part 1. Biochemistry of HIV

HIV has been identified as a retrovirus of the *Lentiviridae* family, existing as two genetically distinct subtypes, denoted as HIV 1 and HIV 2.<sup>2.1, 2.2</sup> HIV 1 is the pathogen of greater consequence.<sup>2.2</sup> HIV 1 replicates, primarily, in the human T4+ lymphocytes.<sup>2.2, 2.5</sup> Replication begins with the binding of a virion to the surface of a lymphocyte.<sup>2.2, 2.5</sup> The virion is internalised by the cell and its RNA genome and retroviral enzymes are

released.<sup>2.2, 2.5</sup> Double stranded DNA is produced from the RNA genome by the action of the retroviral enzymes (including reverse transcriptase) and is incorporated into the host cell genome.<sup>2.2, 2.5</sup> Cellular enzymes then perform the expression of the viral genes, resulting in the synthesis of structural and enzymatic proteins which exist in immature forms within polyproteins.<sup>2.2, 2.5</sup> These immature proteins aggregate at the cell membrane—which begins to bud off—and viral RNA is drawn into the nascent virion.<sup>2.2, 2.5</sup> Maturation of the immature viral proteins is effected by the action of a virally encoded enzyme, the HIV protease (HIV PR).<sup>2.2, 2.5</sup> This completes the replication cycle and the resulting virions are able to infect an adjacent cell.<sup>2.2, 2.5</sup>

The genome of the virus, contained within a dimer of single stranded RNA, consists of three major genes—*gag*, *pol*, and *env*.<sup>2.1, 2.2, 2.5</sup> The *gag* gene encodes structural proteins which require processing by the HIV PR.<sup>2.1, 2.2, 2.5</sup> The *pol* gene contains the transcript for viral enzymes, including the HIV PR, and is translated as the result of a frameshift within *gag* (evasion of the termination codon of *gag*) to give a *gag-pol* polyprotein.<sup>2.1, 2.2, 2.5</sup> Autocleavage of this polyprotein releases the active HIV PR, which then processes other viral proteins contained within the *gag-pol* polyprotein and the structural proteins arising from the *gag* gene.<sup>2.2, 2.6</sup> Finally, the *env* gene encodes the surface proteins of the virion, responsible for binding to the host cell.<sup>2.1</sup>

The proteolytic processing by the HIV PR is vital to viral replication.<sup>2.1, 2.2</sup> Abolition of the activity of the HIV PR by site directed mutagenesis produces mutant virions with unprocessed polyproteins, incapable of infecting new cells.<sup>2.1</sup> This crucial role of the HIV PR makes it a prime target for chemotherapeutic intervention.<sup>2.1, 2.2, 2.5</sup> Consequently,



the HIV PR has been the subject of intense research, culminating in the recent marketing of HIV PR inhibitors, such as Saquinavir, discussed later.<sup>2.1, 2.2, 2.4</sup>

## Part 2. Structural Properties of the HIV PR

The HIV PR belongs to the family of aspartic acid proteases, named for the pair of aspartic acid residues which constitute the catalytic groups in their active sites.<sup>2.2</sup> The family includes some well known non-viral proteases such as pepsin and renin.<sup>2.2</sup>

Although there is minimal sequence homology between the retroviral and the classical (non-viral) proteases, the catalytic triad, Asp-Thr(Ser)-Gly, is highly conserved.<sup>2.1, 2.2</sup> The crystal structure of the HIV PR, shown in Figure 2.1, reveals that it exists as a homodimer with the active site located at the interface of the monomers.<sup>2.7, 2.8</sup> The monomers, each composed of 99 amino acids, are related by a strict two fold axis of rotational symmetry, in which each monomer contributes one catalytic triad to a truly symmetric active site.<sup>2.7, 2.8</sup> The axis of symmetry is perturbed upon the binding of a non-symmetrical inhibitor or substrate.<sup>2.2</sup> In contrast, other aspartic acid proteases exist as monomers of approximately 325 residues. They contain two homologous domains, each of which contributes a catalytic triad to a pseudosymmetrical active site.<sup>2.8</sup>

The HIV PR is characterised by two conformationally flexible loops, one contributed by each monomer (referred to as the “flaps”).<sup>2.9</sup> The “flaps” close around the substrate in the cylindrical substrate binding groove, which accommodates six to seven residues of a substrate or inhibitor.<sup>2.1, 2.9</sup> The amino acids lining the binding groove are symmetrically disposed about the catalytic aspartic acid residues (Asp 25/125), centered in the hydrophobic active site.<sup>2.9</sup> A water molecule, present in all the HIV PR-inhibitor crystal

structures solved to date, is a striking feature not known in other aspartic acid proteases.<sup>2.1, 2.2</sup> This water is hydrogen bonded in a tetrahedral fashion to the amide hydrogens of Ile 50/150 residues in the “flap” and to the carbonyl oxygens of residues in the inhibitor (see Figure 2.2).<sup>2.1, 2.2</sup> It is crucial in bringing together elements of the “flaps” with the inhibitor, ensuring that the inhibitor binds in an extended beta sheet-like conformation.<sup>2.2</sup>

Figure 2.1. The crystal structure of the HIV PR.

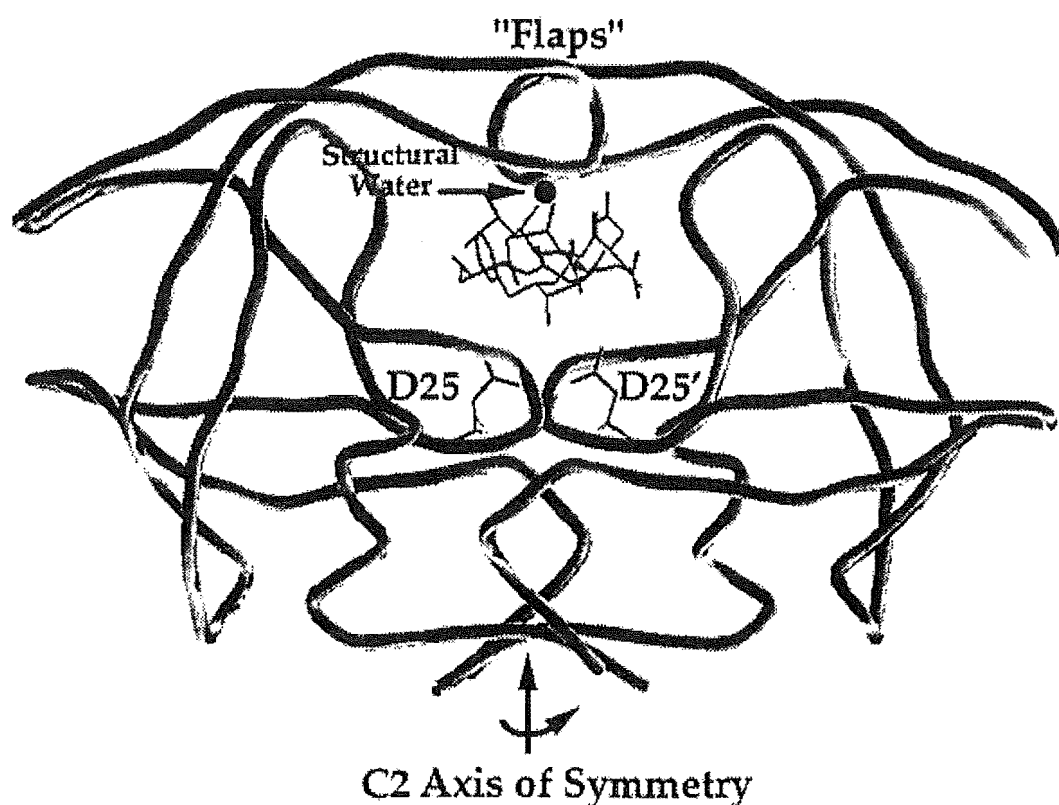
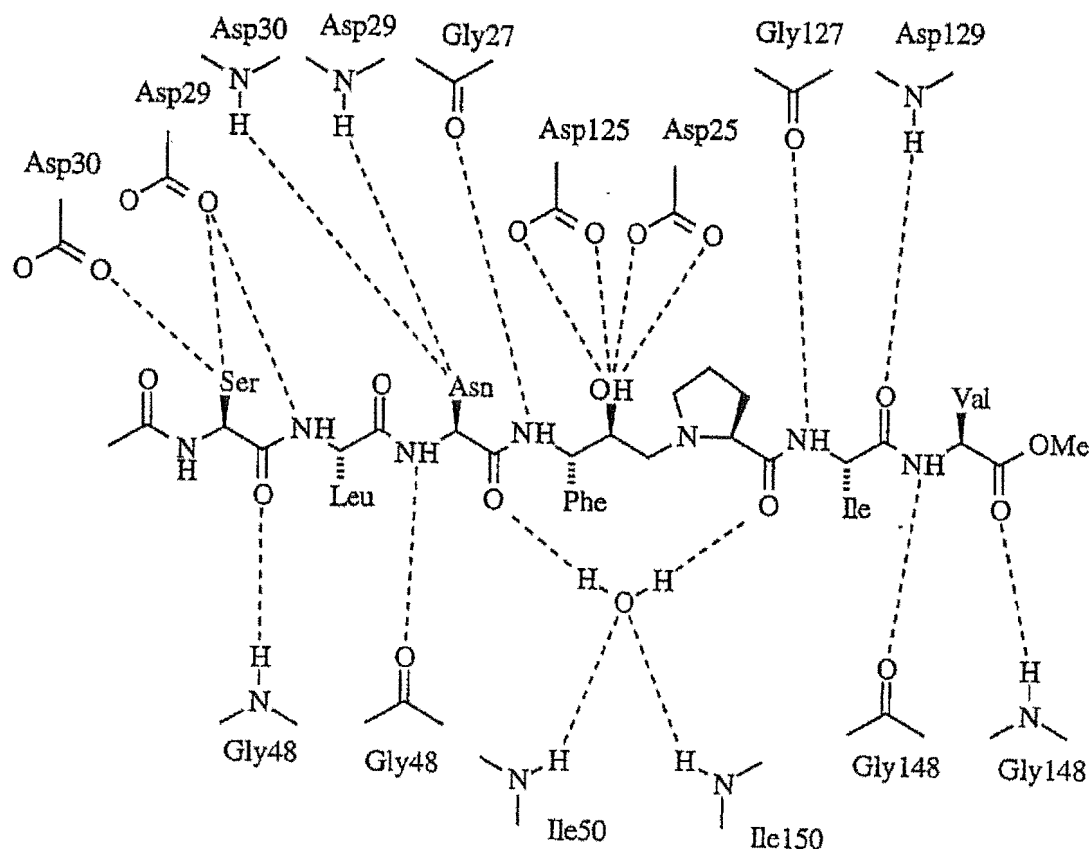


Figure 2.2. Interactions between an inhibitor and the HIV PR, showing the water bridge hydrogen bonded in a tetrahedral fashion to the “flap”.

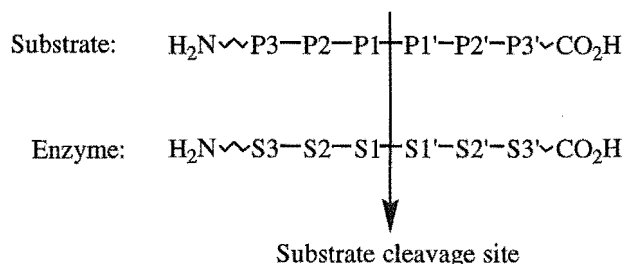


### Part 3. Catalysis by the HIV PR

The HIV PR cleaves its substrates at specific sites, spanning remarkably heterogeneous amino acid sequences.<sup>2.1</sup> At least eight naturally occurring peptide cleavage sites have been identified on the *gag* and *gag-pol* polyproteins, with a variety of amino acids constituting the scissile bond.<sup>2.2, 2.10, 2.11</sup> Consequently, determinants of substrate specificity remain ill-defined.<sup>2.1</sup> However, the occurrence of an aromatic-proline sequence, in three of the processing sites, has been noted with interest, as hydrolysis *N*-terminal to Pro is unusual in mammalian proteases.<sup>2.1</sup> The remaining cleavage sites (Leu-Ala, Met-Met, Phe-Trp, and Phe-Leu) are diverse and reflect a general trend for

hydrophobic residues in the P1 and P1' positions.<sup>2.1, 2.2, 2.11</sup> See Figure 2.3 for definition of P/P' terminology.<sup>2.12</sup>

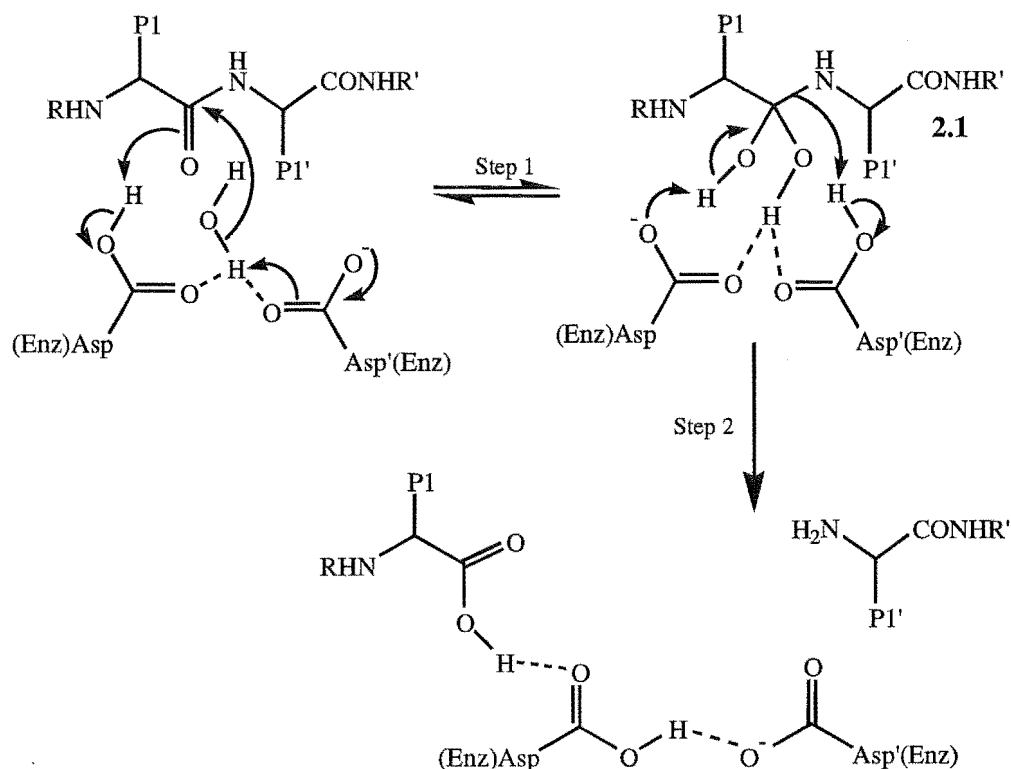
Figure 2.3. Technique for labelling positions flanking the cleavage site.



Several studies have examined the substrate specificity of the HIV PR using oligopeptides which contain the cleavage sites identified in the viral polyproteins.<sup>2.1, 2.2</sup> It was shown that seven residues spanning P4-P3' are required for efficient cleavage.<sup>2.1, 2.2</sup> Small and hydrophobic residues are optimal at P4, with larger residues of variable polarity at P3 and P3'.<sup>2.2</sup> Hydrophobic residues were shown to be favourable at P2 and P2'.<sup>2.2</sup>

The mechanism of catalysis by the HIV PR has been investigated by a number of workers.<sup>2.2</sup> The proposed mechanism, based on isotope effects and crystallographic and kinetic data,<sup>2.13</sup> is shown in Scheme 2.1. The scissile bond is positioned in the active site, such that the negatively charged Asp residue is able to activate a water molecule which adds to the carbonyl group of the amide to be cleaved (Scheme 2.1, Step 1).<sup>2.5, 2.13</sup> The resulting tetrahedral intermediate (2.1) is stabilised by protonation by the other Asp residue. Disassembly of the tetrahedral intermediate (2.1), shown in Step 2, releases the cleaved substrate.<sup>2.5, 2.13</sup>

Scheme 2.1



#### Part 4. Inhibition of the HIV PR

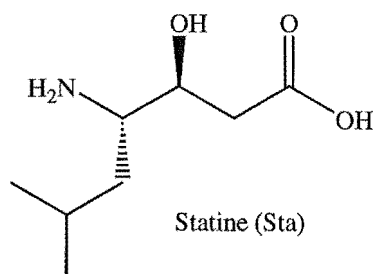
Inhibition of the HIV PR represents a viable strategy for developing antiviral agents.<sup>2.1</sup>

In fact, the HIV PR is an ideal target for chemotherapeutic intervention for the following reasons:<sup>2.6</sup>

- The HIV PR is essential to viral replication.
- Cleavages performed by the HIV PR are not mediated by cellular proteases, such that the HIV PR is sufficiently distinct from cellular proteases to allow selective inhibition.
- The solid knowledge base on other aspartic acid proteases has been extrapolated to studies on the HIV PR.
- The HIV PR has been characterised and can be produced by chemical synthesis or by expression of the cloned gene. Rapid assays exist to screen potential inhibitors.

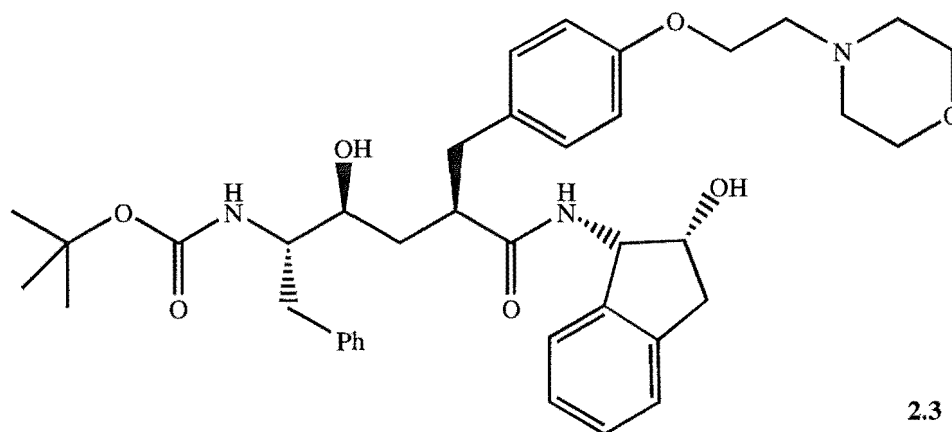
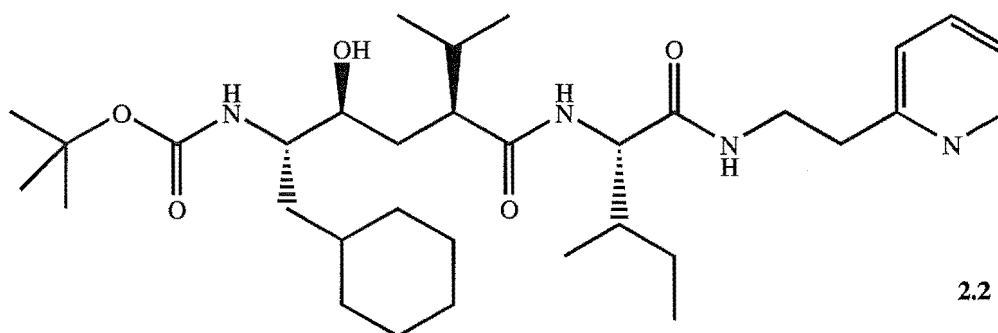
The new combination therapies, which attack the virus on several different fronts, including by inhibition of the HIV PR, have worked well, even reducing the level of the virus to undetectable levels in some patients.<sup>2,4</sup> However, administration of a combination therapy involves a complicated schedule of drug taking, together with dreadful side effects, making it impractical for many HIV patients.<sup>2,4</sup> Further, failure of patients to adhere to the complex treatment, gives the virus a perfect opportunity to develop resistance.<sup>2,4</sup> Therefore, there is still a very real need for the development of new HIV drug therapies, including improved HIV PR inhibitors, to combat this deadly virus.<sup>2,4</sup>

A classical approach to enzyme inhibition, the incorporation of non-hydrolysable transition state mimics into substrate analogues, has generated a number of potent HIV PR inhibitors.<sup>2.1, 2.2</sup> Such peptidomimetics possess  $sp^3$  hybridisation at the central carbon, posing for the scissile carbonyl of the substrate, thereby mimicking the tetrahedral intermediate (**2.1**, Scheme 2.1).<sup>2.2</sup> An early example, pepstatin A (a known inhibitor of the aspartic acid protease renin) was shown to be an inhibitor of the HIV PR ( $K_i=400$ -1400 nM).<sup>2.1, 2.2</sup> Pepstatin A (*N*-isoValeryl-Val-Val-Sta-Ala-Sta) possesses the unusual amino acid statine (Sta) which mimics the tetrahedral intermediate (**2.1**).<sup>2.1, 2.2</sup>



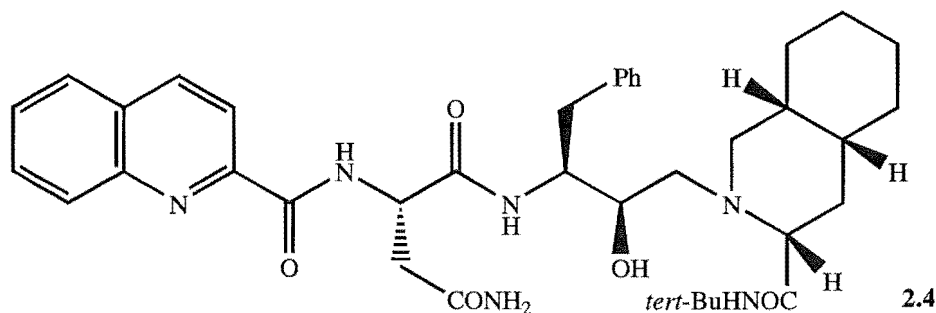
To date, hydroxyethylene-based peptidomimetics have been used extensively, resulting in some potent inhibitors of the HIV PR.<sup>2.2</sup> Two examples, the tripeptide mimics Ro-31-8588 (**2.2**) and L-689,502 (**2.3**) are notable for their subnanomolar inhibition constants

( $K_i=0.3$  and  $0.45$  nM, respectively).<sup>2,10</sup> The crystal structures of each of these inhibitors (**2.2** and **2.3**) complexed with the HIV PR have been solved.<sup>2,10</sup> In both cases, the hydroxyl group of the inhibitor is centered about the two active site Asp carboxylates of the enzyme.<sup>2,10</sup> Interestingly, the *tert*-butoxy groups at the *N*-termini of the inhibitors (**2.2** and **2.3**) occupy the S2 subsite of the enzyme and are critical for potency (see Figure 2.3 for definition of S terminology<sup>2,12</sup>).<sup>2,10</sup>



In 1990 Roberts and co-workers reported the design of very potent and selective inhibitors of the HIV PR containing a hydroxyethylamine transition state mimic.<sup>2,2</sup> One such compound Ro-31-8959 (**2.4**) was the first protease inhibitor to be administered to humans. Compound **2.4** is known as Saquinavir and is now in clinical use for the treatment of HIV infection ( $IC_{50}<0.4$  nM).<sup>2,10</sup> In contrast to other hydroxyl containing

inhibitors, such as **2.2** and **2.3**, the *R*-stereoisomer of the secondary alcohol of **2.4** provides the more potent inhibitor.<sup>2.2, 2.10</sup> The decahydro-isoquinoline group of **2.4** substitutes for the proline associated with the S1' binding pocket and contributes considerably to potency.<sup>2.2, 2.9, 2.10</sup> The corresponding derivative containing proline in this position is 50-fold less potent, suggesting that the decahydro-isoquinoline group encourages the inhibitor into a conformation recognised by the enzyme.<sup>2.2</sup> Further, structure-activity correlations suggest that Phe occupies the S1 subsite of the enzyme, Asn occupies the S2 subsite, the *tert*-butylamide occupies S2', and the terminal quinoline fills the S3 subsite.<sup>2.9</sup>



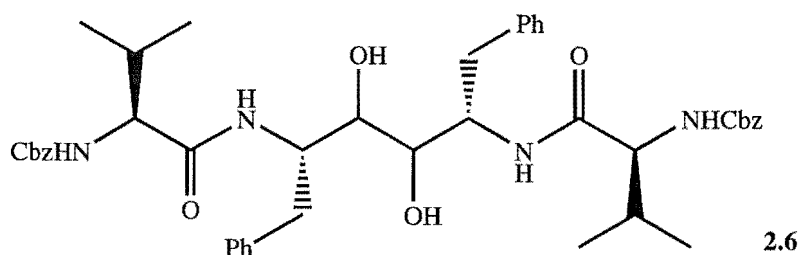
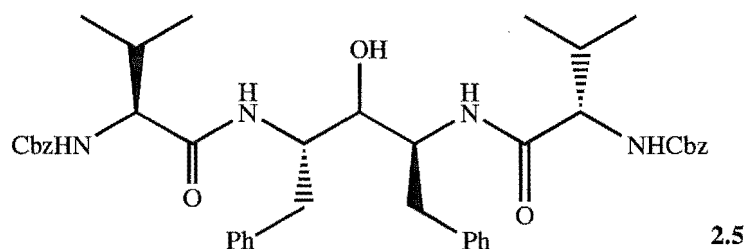
A further class of HIV PR inhibitors possess elements of C<sub>2</sub> rotational symmetry, capitalising on the unique symmetry of the dimeric enzyme.<sup>2.1, 2.2, 2.10, 2.14</sup>

Pseudosymmetric inhibitors represent an important advance in HIV PR inhibition since they are of diminished peptide character, leading to greater metabolic stability.<sup>2.2, 2.14</sup>

Further, they confer high specificity for retroviral proteases, over mammalian aspartic acid proteases, whose binding sites are less symmetric.<sup>2.2, 2.14</sup> In an example of a pseudosymmetric inhibitor, A-74704 (**2.5**) (IC<sub>50</sub>=3 nM), the C-O bond of the central alcohol comprises the C<sub>2</sub> axis of symmetry.<sup>2.2</sup> A crystal structure reveals that **2.5** binds to the HIV PR in a symmetrical fashion.<sup>2.15</sup> A water molecule (present in all the HIV PR-inhibitor crystal structures solved to date<sup>2.1, 2.2</sup>) is hydrogen bonded to the carbonyl

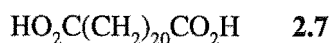


oxygen atoms of the Val residues of **2.5** and to the amide hydrogens of the Ile 50/150 residues in the “flap” region of the protease.<sup>2.15</sup> The S1 and S2 subsites of the enzyme bind the benzyl (Phe) and the isopropyl (Val) groups of the inhibitor (**2.5**).<sup>2.15</sup> A second example of a pseudosymmetrical inhibitor is provided by the diol (**2.6**), where the C2 axis bisects the carbon-carbon bond of the diol.<sup>2.14</sup> Diol inhibitors, such as **2.6**, are approximately an order of magnitude more potent than inhibitors of the type **2.5**.<sup>2.14</sup> Surprisingly, the activity of compound **2.6** is reasonably insensitive to the stereochemistry of the hydroxyl groups ( $IC_{50}=0.22-0.38$  nM).<sup>2.14</sup> As predicted, the inhibitory activity of compounds **2.5** and **2.6** is selective for the retroviral proteases.<sup>2.14, 2.15</sup>



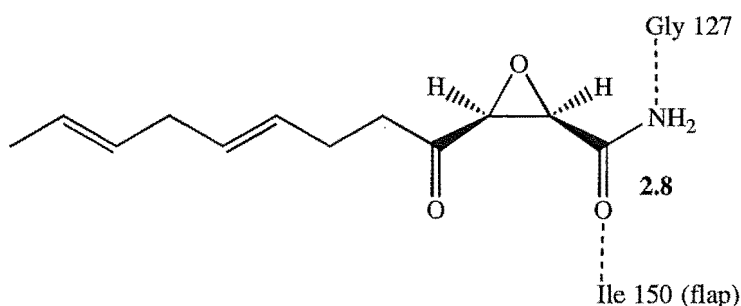
As discussed in the introduction to this thesis, a major strategy to reduce pharmacological problems is the development of inhibitors which mimic bioactive peptides but have either reduced or no peptide character.<sup>2.9</sup> The dicarboxylate (**2.7**) was among the first reported non-peptide inhibitors of the HIV protease ( $IC_{50}=12$   $\mu$ M).<sup>2.16</sup> The result is significant since only two potential enzyme binding sites are present.<sup>2.16</sup> It is proposed that the carboxylate side chains of **2.7** engage the two Arg residues located at either end of the

substrate binding groove of the enzyme.<sup>2.16</sup> This is supported by the importance of the CH<sub>2</sub> chain length. Compounds analogous to **2.7**, but with an insufficient chain length for the CO<sub>2</sub>H groups to interact with the Arg residues, are significantly less active.<sup>2.16</sup>



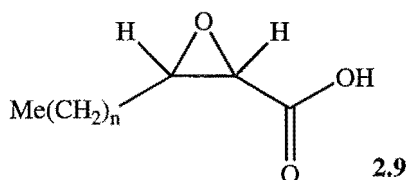
A number of epoxide containing peptidomimetics have been shown to inactivate the HIV PR.<sup>2.1, 2.2, 2.17, 2.18, 2.19, 2.20, 2.21, 2.22</sup> For example, cerulenin (**2.8**, Figure 2.4), a naturally occurring antibiotic, weakly inhibits the HIV (IC<sub>50</sub>=2500 μM) and other aspartic acid proteases.<sup>2.17</sup> Structure-activity studies, performed on **2.8**, suggest that inhibitory action occurs at the active site of the enzyme.<sup>2.17</sup> Modelling studies demonstrated that the terminal amide of **2.8** can form hydrogen bonds to the enzyme, which position the 4-oxo group in the place of the carbonyl group of the scissile bond of the substrate.<sup>2.17</sup> This places the epoxide close to the position occupied by the amide nitrogen of the substrate (Figure 2.4).<sup>2.17</sup> Catalytic action on the inhibitor, thus placed, would lead to esterification of one of the active site residues and hence enzyme inactivation.<sup>2.17</sup>

Figure 2.4



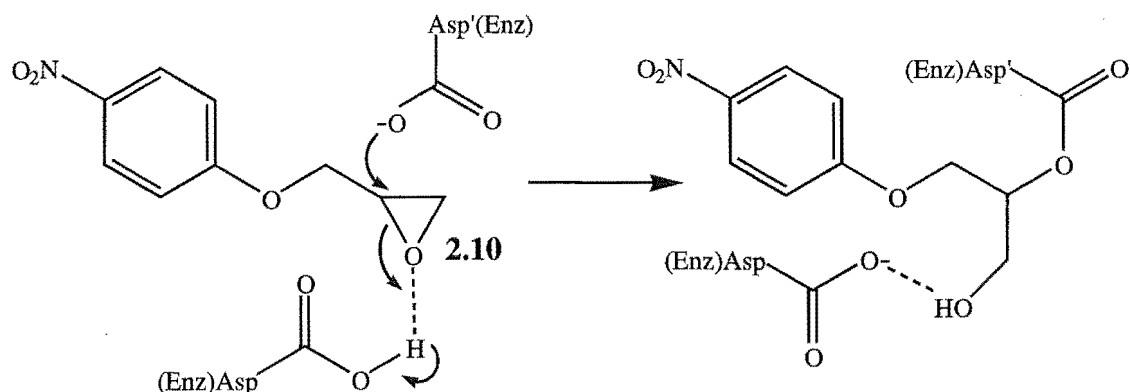
Cerulenin (**2.8**) is cytotoxic and therefore unsuitable for clinical use.<sup>2.17</sup> However, **2.8** has provided a lead structure for development of HIV inhibitors of potential therapeutic use. For example, Blunenstein *et al*<sup>2.18</sup> studied related analogues of **2.8**, such as **2.9**, and suggested that the length of the hydrophobic residue is important.<sup>2.18</sup> Compound **2.9**

( $n=8$ ), having the same chain length as cerulenin (**2.8**), was found to be a weak inhibitor, whereas longer chain lengths led to diminished activity.<sup>2.18</sup> Further, the epoxide was shown to be essential for inhibitory activity.<sup>2.18</sup> Compounds analogous to **2.9** lacking the epoxide function were inactive.<sup>2.18</sup>

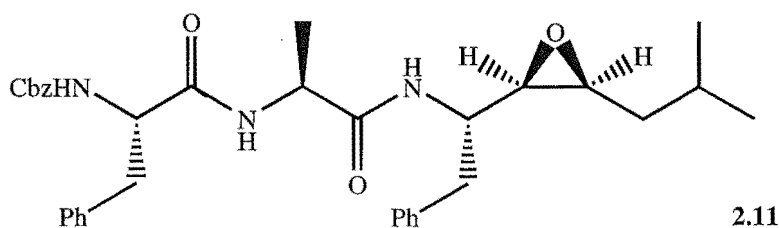


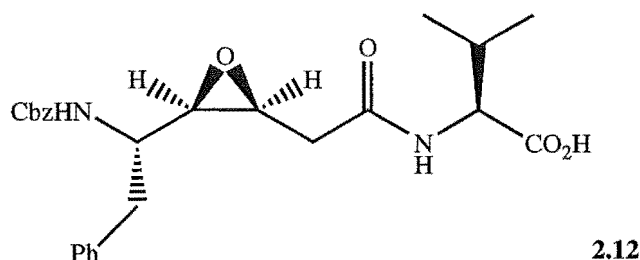
Another epoxy-containing peptidomimetic, 1,2-epoxy-3-(*p*-nitrophenoxy)propane (ENP) (**2.10**, Scheme 2.2), is an irreversible inhibitor of aspartic acid proteases, including the HIV 1 PR ( $K_i=9.9$  mM).<sup>2.2, 2.19</sup> The crystal structure of **2.10** bound to the Simian Immunodeficiency Virus protease (SIV PR) has been determined (SIV is a retrovirus closely related to HIV).<sup>2.19</sup> This was the first reported structure of a retroviral protease bound to a covalent inhibitor.<sup>2.19</sup> The major interaction between the protease and the inhibitor (**2.10**), the covalent bond to one of the active site Asp residues, is shown in Scheme 2.2.<sup>2.19</sup> Scheme 2.2 details the proposed mechanism of inactivation, where the negatively charged Asp residue attacks the epoxide to form an ester linkage.<sup>2.19</sup> Further, the crystal structure revealed that the phenyl group of **2.10** occupied the S1 subsite of the enzyme and that the nitro group interacted with the “flap”.<sup>2.19</sup> The conserved water molecule was hydrogen bonded to amide hydrogens in the “flap”.<sup>2.19</sup>

Scheme 2.2

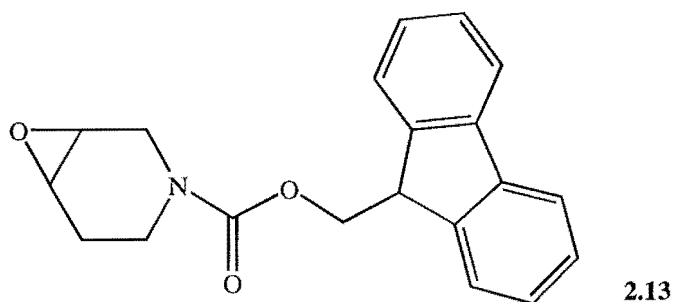


Compound **2.10** provides a lead structure for the design of irreversible inhibitors of the HIV PR. For example, salient structural features of **2.10** were incorporated into a tripeptide analogue (containing residues typically found in substrates) giving compound **2.11**, an irreversible inhibitor of the HIV PR ( $K_i=20\ \mu\text{M}$ ).<sup>2,20</sup> Inactivation by **2.11** is thought to result from specific alkylation of a single active site Asp residue, analogous to the mode of action of compound **2.10** (Scheme 2.2).<sup>2,20</sup> It is presumed that the P3-P1' groups of **2.11** occupy the S3-S1' substrate binding sites of the enzyme.<sup>2,20</sup> Compound **2.12** provides a further example of a potent and selective irreversible inhibitor of the HIV PR ( $K_i=1.32\ \mu\text{M}$ ).<sup>2,21</sup> The *cis* stereochemistry of the epoxide was critical to the potency of compound **2.12**.<sup>2,21</sup> An analogous compound with a *trans* epoxide demonstrated much poorer activity.<sup>2,21</sup> Both compounds **2.11** and **2.12** contain the (*R,S*)-isomer of the *cis* epoxide.<sup>2,21, 2,20</sup>





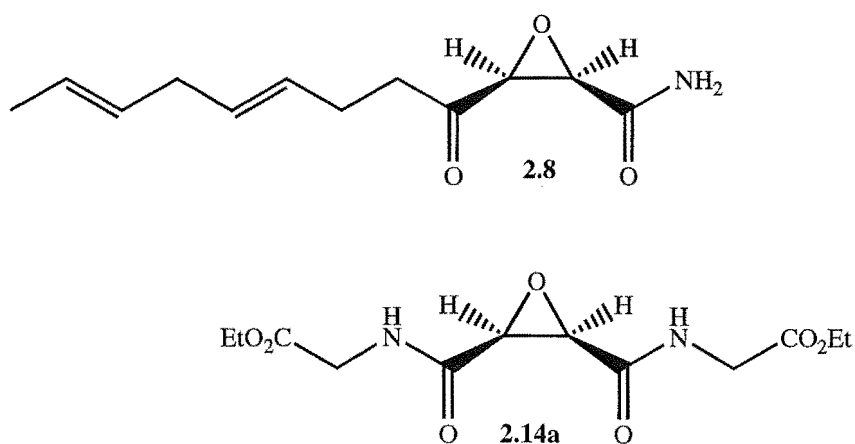
A recent paper details the investigation of a number of mono, di and tri substituted, non peptidic, epoxides of which the *cis*-1,2-disubstituted epoxide (**2.13**) proved to be the most effective inhibitor ( $K_i=65 \mu\text{M}$ ).<sup>2.22</sup> Inhibition is irreversible and results from covalent attachment of compound **2.13** to a catalytic Asp residue (shown by mass spectrometric sequencing of labelled protolytic peptides).<sup>2.22</sup> Interestingly, similar *trans*-1,2-disubstituted epoxides did not detectably inhibit the HIV PR and it was suggested that this was due to steric hindrance to backside attack at the epoxide, by the unprotonated Asp residue of the enzyme (see Scheme 2.2 for the proposed mechanism of inactivation by irreversible epoxide inhibitors such as compound **2.10**).<sup>2.22</sup>

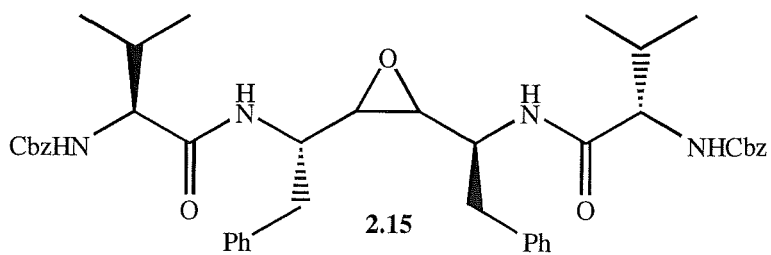
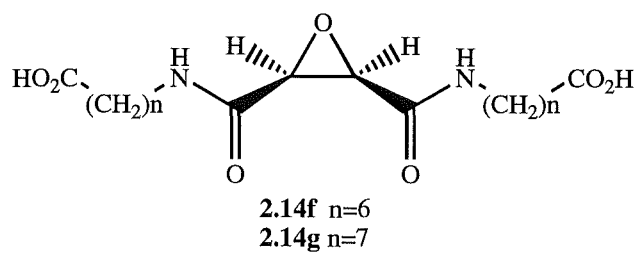
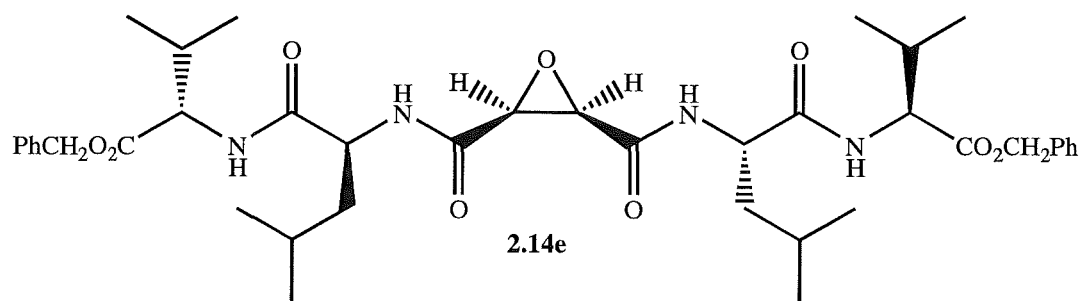
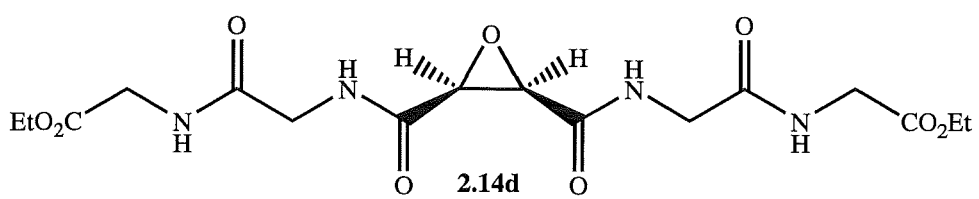
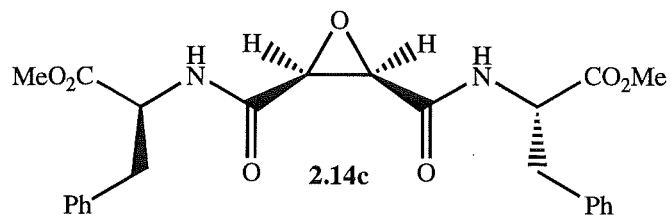
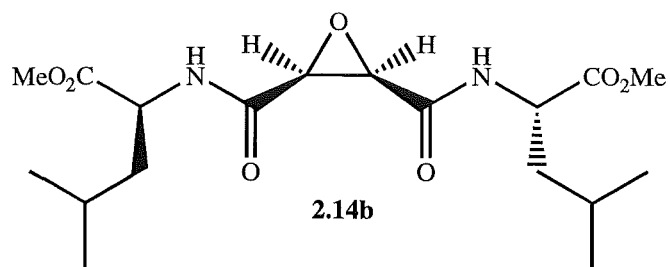


## Results and Discussion

### Part 1. Pseudosymmetrical Potential Inhibitors of the HIV PR

This section presents a series of compounds (**2.14a-g**) designed as potential inhibitors of the HIV PR. Compounds **2.14a-g** incorporate, in a pseudosymmetric compound, the elements of cerulenin (**2.8**) considered to be important for inhibition (the amide and epoxide moiety). As discussed in the introduction to this chapter, a number of epoxy containing peptidomimetics, such as **2.8**, have been shown to be inhibitors of the HIV PR.<sup>2.17, 2.18, 2.19, 2.20, 2.21, 2.22</sup> Further, peptidomimetics possessing elements of C<sub>2</sub> rotational symmetry, such as **2.5** (discussed in the introduction to this chapter), have proved to be potent and selective inhibitors of the HIV PR.<sup>2.1, 2.2, 2.14, 2.15</sup> In an attempt to develop epoxide-containing inhibitors, this concept of symmetry was utilised to design the novel structures (**2.14a-g**). Interestingly, a similar approach was adopted independently by Park and co-workers to give compound **2.15**, an epoxy-containing pseudosymmetrical inhibitor of the HIV PR ( $K_i=75$  nM).<sup>2.23</sup>





It was anticipated that the configuration of the epoxide would be important to the design of the peptidomimetics (**2.14a-g**). The *cis* stereochemistry was adopted, since other epoxides shown to be inhibitors of the HIV PR, such as **2.11**, **2.12**, and **2.13** (discussed in the introduction to this chapter) all contain *cis* epoxides.<sup>2.20, 2.21, 2.23</sup> In fact the *cis* configuration of the epoxide was shown to be critical to the potency of compound **2.12**.<sup>2.21</sup> Consideration of appropriate elements to enhance enzyme recognition led to a series of peptide-based compounds (**14a-e**), which contain a pair of amino acids or dipeptides, similar to known inhibitors such as **2.15**.<sup>2.14, 2.15, 2.23</sup> A pair of non-peptide compounds (**2.14f** and **2.14g**), which contain a long CH<sub>2</sub> chain, arose from consideration of the dicarboxylate (**2.7**), also discussed in the introduction to this chapter.<sup>2.16</sup>

#### *Peptide-Based Potential Inhibitors of the HIV PR*

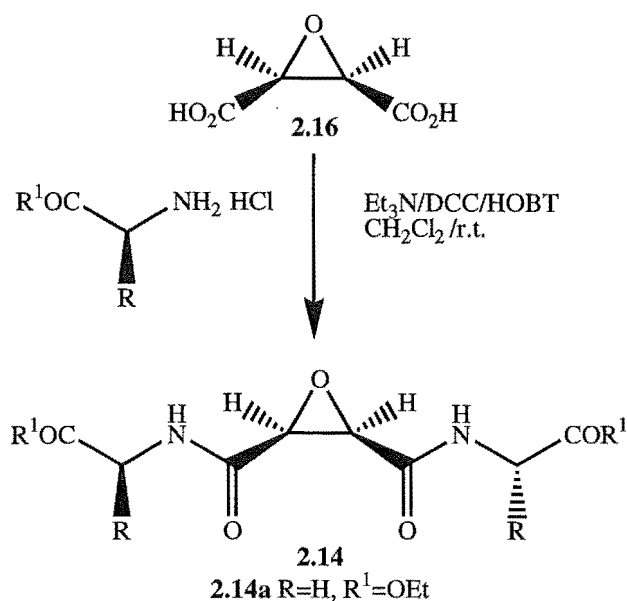
As discussed in the introduction to this chapter, a number of peptide-based molecules have been reported as inhibitors of the HIV PR.<sup>2.14, 2.15</sup> Since the enzyme forms a network of hydrogen bonds and hydrophobic interactions to a substrate sequence, inhibitors containing appropriate amino acid residues enhance recognition and binding affinity.<sup>2.1</sup> Here, a series a novel peptide-based epoxides (**2.14a-e**) are reported.

Initial work in this area concentrated on the introduction of a single amino acid to give compound **2.14a** (Scheme 2.3). As shown in Scheme 2.3, this was achieved by the dicyclohexylcarbodiimide (DCC) mediated coupling<sup>2.24</sup> of Gly ethyl ester hydrochloride with compound **2.16** (synthesised by a literature procedure<sup>2.25</sup>). Compound **2.14a** was regarded as a model to demonstrate the feasibility of coupling a pair of amino acids with



**2.16** in a single step. An appropriate hydrophobic amino acid was required to enhance enzyme recognition and binding affinity.

Scheme 2.3



The consideration of an appropriate amino acid for incorporation into compound **2.14** involved some speculation as to the potential binding mode of **2.14** to the HIV PR. Modelling studies on cerulenin (**2.8**) indicated that the amide is hydrogen bonded to Ile 150 in the “flap” and to Gly 127, and that the 4-oxo group is close to the catalytic Asp residues—(see Figure 2.4).<sup>2.17</sup> This suggests that compounds of the type **2.14** could bond to the enzyme in a similar fashion to that shown in Figure 2.5. Here the proposed interactions (with the catalytic Asp residues, Ile 150, and Gly 127) are identical to those proposed for cerulenin (**2.8**).<sup>2.17</sup> Crystallographic data<sup>2.1, 2.9</sup> on HIV PR-inhibitor complexes suggests that this would place the P2' group in a position to bind in the S2' subsite. Further, the amide between the P1 and P2 residues may coordinate to Ile 50 and Gly 27, thus positioning the P1 and P2 groups to bind in the S1 and S2 subsites. However, a symmetrical binding mode may be adopted since it has been demonstrated

that symmetric inhibitors, such as compound **2.5** (discussed in the introduction to this chapter) generally interact with the enzyme in a symmetric fashion.<sup>2.15, 2.26</sup>

Figure 2.4

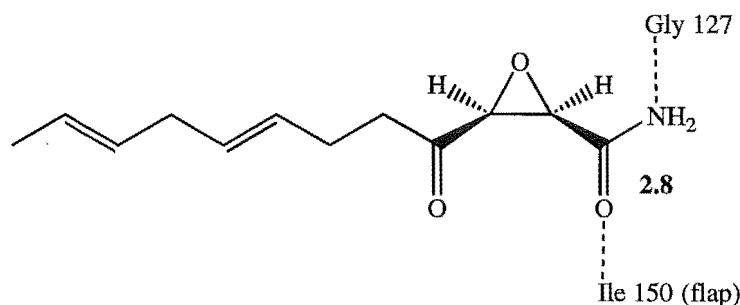
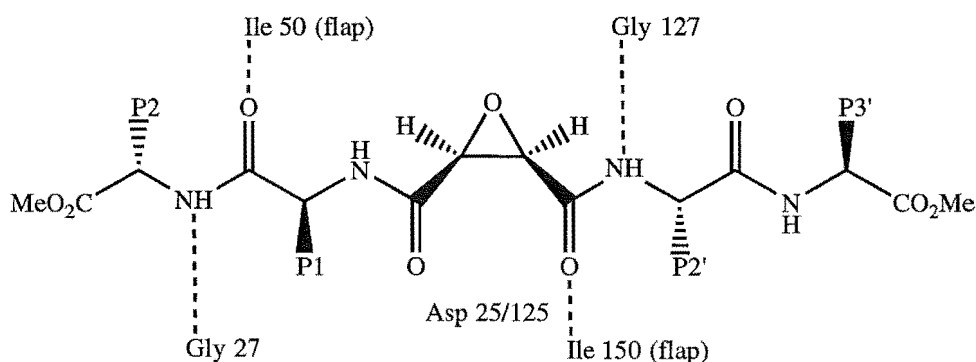


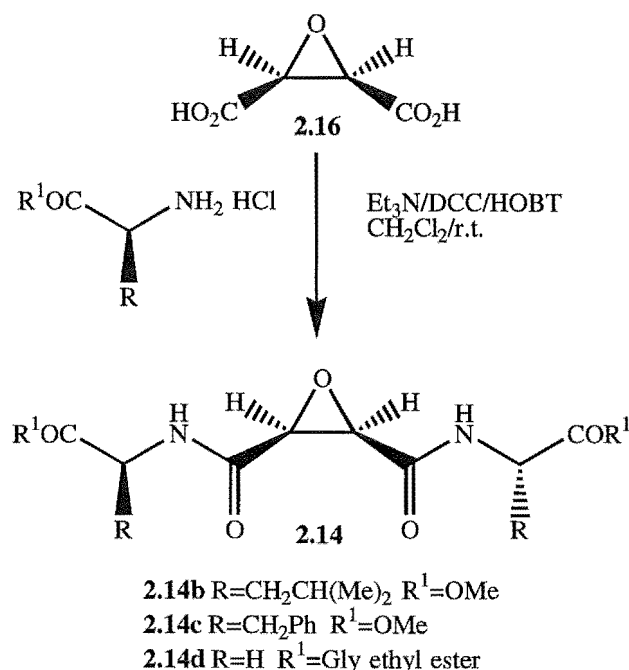
Figure 2.5



To enhance enzyme recognition, suitable P1, P2, and P2' amino acids were investigated. L-Leu methyl ester hydrochloride was coupled<sup>2.24</sup> with **2.16** to give compound **2.14b** (see Scheme 2.3 for a general scheme). L-Leu methyl ester hydrochloride was chosen since naturally occurring peptide cleavage sites with Leu in the P1 position have been identified<sup>2.2</sup> and further Leu is a acceptable residue at both P2' and at P2. Thus, for the mode of binding suggested in Figure 2.5, Leu is an suitable residue to enhance enzyme recognition. Further work in this area involved the coupling<sup>2.24</sup> of L-Phe methyl ester hydrochloride with **2.16** to give compound **2.14c** (see Scheme 2.3 for a general scheme). HIV PR catalysed cleavage of peptides with Phe in the P1 position is extremely

common.<sup>2.1, 2.2</sup> Phe is also known to occur in the P2 position<sup>2.2</sup> and is, therefore, also an acceptable residue to enhance enzyme recognition.

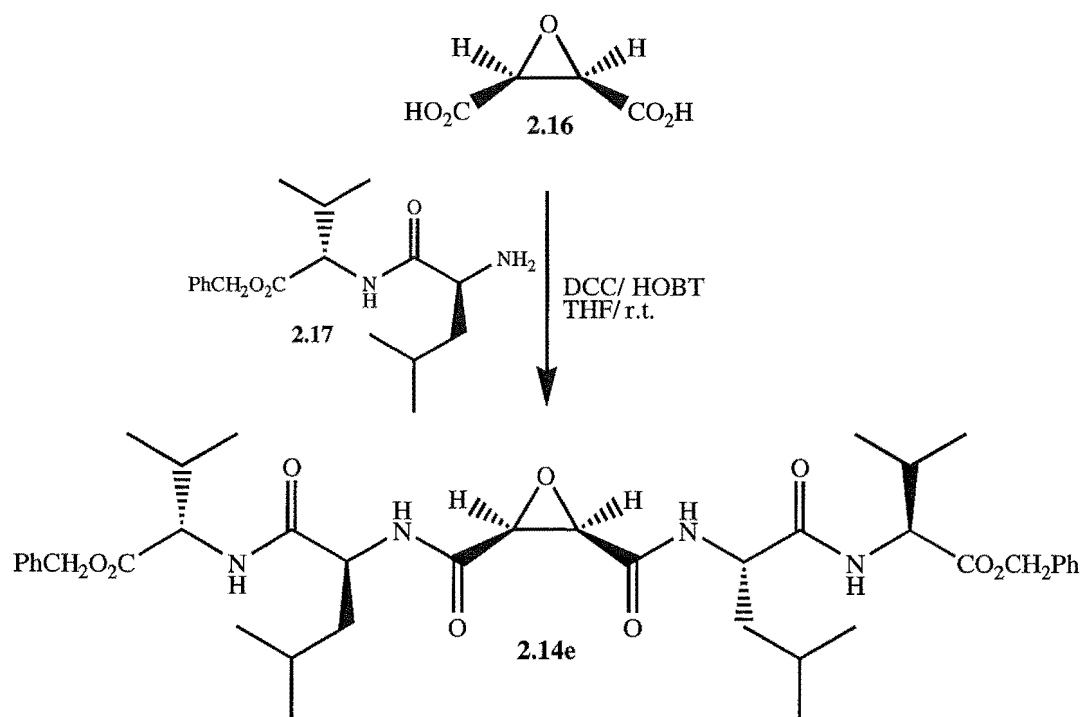
Scheme 2.3



In an attempt to further enhance enzyme recognition, the coupling<sup>2.24</sup> of a dipeptide with compound **2.16** was investigated. The enhanced activity of the “tetrapeptide” (**2.5**) over an equivalent “dipeptide” analogue (discussed in the introduction to this chapter) attests the importance of extra residues to enhance recognition and binding affinity.<sup>2.2</sup> Gly-Gly ethyl ester hydrochloride, chosen for initial investigations as it was commercially available, was coupled<sup>2.24</sup> with **2.16** giving a compound, tentatively assigned to the structure **2.14d** (see Scheme 2.3 for a general scheme). Although purification of **2.14d** proved difficult due to its solubility properties (discussed later), the reaction demonstrated the feasibility of the coupling of a dipeptide with compound **2.16**.

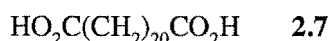
Suitable residues for incorporation into a “tetrapeptide” example of compound **2.14** were selected on the basis of known substrate specificity of the HIV PR<sup>2,2</sup> and the possible binding mode of compounds of the type **2.14**, shown in Figure 2.5. As discussed above, Leu is a suitable P1 and P2' amino acid,<sup>2,1, 2,2</sup> Further Val is a suitable residue at the P2 and P3' positions.<sup>2,1, 2,2</sup> L-Leu-L-Val benzyl ester (**2.17**) was coupled<sup>2,27</sup> with **2.16** to give compound **2.14e** as shown in Scheme 2.4. The coupling was performed on the free amine (**2.17**), as this resulted in a cleaner product than that obtained from the coupling of the formate salt of **2.17** with compound **2.16**.

Scheme 2.4



*Non-Peptide-Based Potential Inhibitors of the HIV PR*

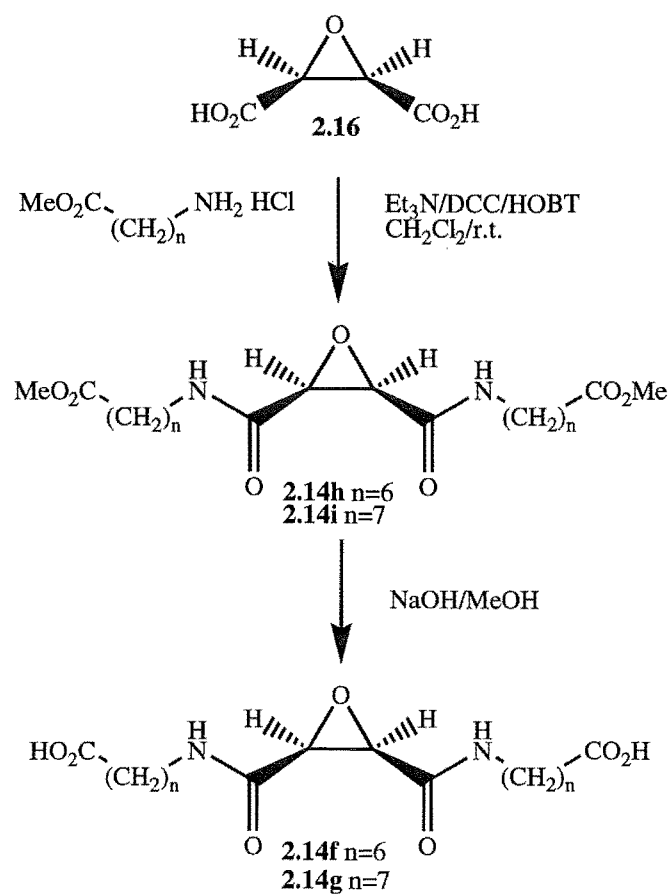
Since peptide inhibitors are traditionally associated with problems of low bioavailability, the development of non-peptide inhibitors of the HIV PR is of considerable therapeutic importance.<sup>2.16</sup> As discussed in the introduction to this chapter, the dicarboxylate (**2.7**) has been reported as a weak non-peptide inhibitor of the HIV PR.<sup>2.16</sup> Binding to the HIV PR is thought to occur between the carboxylate groups of compound **2.7** and the Arg 8/108 residues located at the end of the substrate binding groove of the enzyme.<sup>2.16</sup> The potential inhibitors (**2.14f** and **2.14g**, Scheme 2.5) were designed to incorporate carboxylate groups of appropriate separation to engage the Arg residues (20 or 22 bonds, since in compound **2.7** the carboxylates are separated by 21 bonds<sup>2.16</sup>).



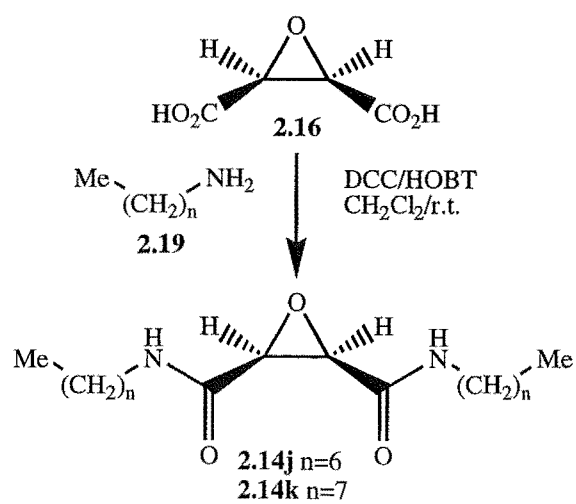
As shown in Scheme 2.5, coupling<sup>2.24</sup> of the amines (**2.18**, n=6/7) (synthesised by a literature procedure<sup>2.28, 2.29</sup>) with compound **2.16** gave compounds **2.14h** and **2.14i**. Base catalysed hydrolysis of the methyl esters of **2.14h** and of **2.14i** gave the target compounds (**2.14f** and **2.14g**, Scheme 2.5).

Further work in this area involved the coupling<sup>2.24</sup> of **2.16** with the amines (**2.19**, n=6/7) to give compounds **2.14j** and **2.14k** (Scheme 2.6). Compounds **2.14j** and **2.14k** do not have carboxylate groups (as in **2.14f** and **2.14g**) to enhance inhibitor activity by binding to the Arg residues. They would, therefore, be expected to be weaker inhibitors than **2.14f** and **2.14g**, acting as “controls” to demonstrate the role of the carboxylate groups in binding to the enzyme. However, since compounds **2.14f** and **2.14g** were inactive (discussed later) the possible role of the carboxylates was unable to be elucidated.

Scheme 2.5

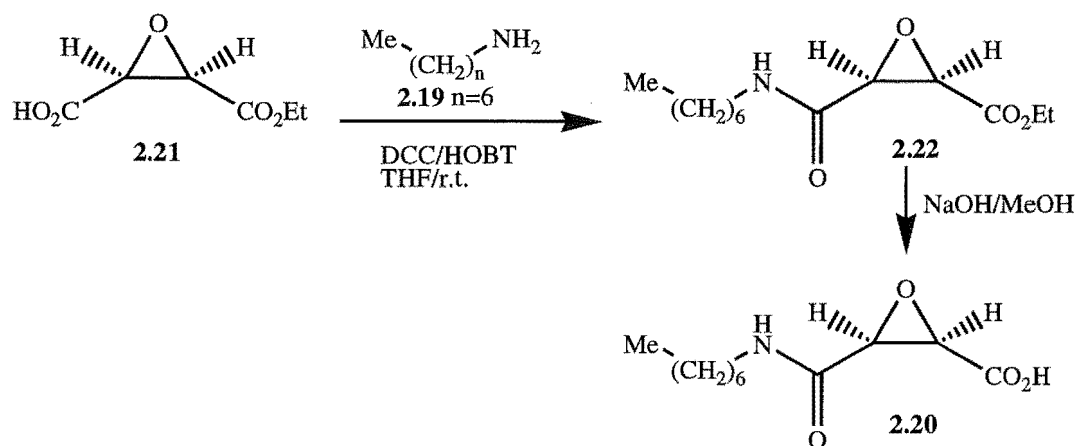


Scheme 2.6



Finally, compound **2.20** (Scheme 2.7) was designed to investigate the importance of the symmetrical nature of compounds such as **2.14j** and **2.14k**. Compound **2.20** is a non-symmetrical analogue of compound **2.14j**. A comparison of the *in vitro* activities of compound **2.20** and compound **2.14j** is presented in Part 3 of this chapter. Compound **2.20** was synthesised as shown in Scheme 2.7. The half ester (**2.21**), synthesised by a literature procedure,<sup>2,30</sup> was coupled<sup>2,27</sup> with heptylamine (**2.19**,  $n=6$ ) to give the intermediate (**2.22**). Base catalysed hydrolysis of **2.22** gave compound **2.20**.

Scheme 2.7



*Structure Assignment of Compounds 2.14a-k***COMPOUND 2.14a**

Compound **2.14a** was purified by recrystallisation to give white crystals. In the  $^1\text{H}$  NMR spectrum of **2.14a**, the resonances due to the  $\text{CH}_2\text{NH}$  protons appeared as separate doublets of doublets at  $\delta$  4.14 and  $\delta$  3.97, while the epoxide protons gave rise to a singlet at  $\delta$  3.76. The  $^{13}\text{C}$  NMR spectrum of **2.14a** was assigned by comparison to that of the Gly ethyl ester cation.<sup>2,31</sup> A mass spectrum and micro analysis were consistent with the proposed structure of **2.14a**.

**COMPOUND 2.14b**

Compound **2.14b** was purified by column chromatography to give a colourless oil. Due to the presence of two stereocentres, compound **2.14b** does not contain a plane of symmetry and therefore magnetic non-equivalence is observed in its NMR spectra. For example, in the  $^1\text{H}$  NMR spectrum of **2.14b**, separate signals were observed for each methyl ester ( $\delta$  3.73 and  $\delta$  3.72) and for each of the  $\text{NH}$  groups ( $\delta$  6.65 and  $\delta$  6.58). In the  $^{13}\text{C}$  NMR spectrum of **2.14b** most carbons gave separate signals. For example, the epoxide carbons appeared at  $\delta$  55.0 and  $\delta$  54.9 and the  $\text{CONH}$  signals at  $\delta$  165.1 and  $\delta$  165.0. These signals were assigned by comparison to the  $^{13}\text{C}$  NMR spectrum of **2.14a**. Other  $^{13}\text{C}$  NMR signals were assigned by comparison to the  $^{13}\text{C}$  NMR spectrum of the Leu methyl ester cation.<sup>2,31</sup>

**COMPOUND 2.14c**

Compound **2.14c** was purified by radial chromatography to give a colourless oil. Attempts to initiate crystallisation were unsuccessful. Compound **2.14c** does not contain



a plane of symmetry and thus magnetic non-equivalence is evident in its NMR spectra. For example, in the  $^1\text{H}$  NMR spectrum of **2.14c**, separate signals were observed for the methyl esters at  $\delta$  3.70 and  $\delta$  3.66 and in the  $^{13}\text{C}$  NMR spectrum most carbons gave rise to separate resonances. A heteronuclear multiple quadrupole correlation (HMQC) experiment was performed to distinguish between the  $\underline{\text{C}}\text{HCH}_2$  and the  $\text{OMe}$  carbons. The signals at  $\delta$  52.4 and  $\delta$  52.3 correlated to the  $^1\text{H}$  NMR methyl ester signals, while the signals at  $\delta$  53.4 and  $\delta$  53.3 correlated to the  $^1\text{H}$  NMR  $\underline{\text{C}}\text{HCH}_2$  multiplet. Other  $^{13}\text{C}$  NMR signals were assigned by comparison to those of **2.14a**, **2.14b**, and the Phe methyl ester cation,<sup>2,31</sup> as appropriate.

#### COMPOUND **2.14d**

The poor solubility properties of **2.14d** in organic solvents made isolation and purification difficult. This compound proved to be soluble in water and methanol, and insoluble in organic solvents. Thus, the standard work-up procedure<sup>2,24</sup> was not feasible. Instead, the evaporated reaction mixture was adsorbed onto silica and eluted with ethyl acetate. Further purification by recrystallisation was unsuccessful. The structure of **2.14d** was suggested on the basis of NMR data. Homonuclear decoupling experiments were performed to assign the  $^1\text{H}$  NMR spectrum of **2.14d**. The signals at  $\delta$  4.18 and  $\delta$  1.26 were shown to be coupled by irradiation and were assigned to the ethyl ester. One of the  $\text{CH}_2\text{NH}$  groups was assigned on the basis of an irradiation at  $\delta$  4.71 ( $\underline{\text{C}}\text{H}_2\text{NH}$ ), which led to collapse at  $\delta$  7.25 ( $\text{CH}_2\text{NH}$ ) (to a broad singlet). The other  $\text{CH}_2\text{NH}$  group was tentatively assigned to signals at  $\delta$  7.73 ( $\text{CH}_2\text{NH}$ ) and the multiplet at  $\delta$  4.00 ( $\underline{\text{C}}\text{H}_2\text{NH}$ ). Irradiation of these signals was inconclusive. The epoxide protons appeared as a singlet at  $\delta$  3.96. The  $^{13}\text{C}$  NMR spectrum of **2.14d** was readily assigned by

comparison to that of **2.14a**. Attempts to obtain a mass spectrum on **2.14d** were unsuccessful.

### COMPOUND **2.14e**

Table 2.1. Homonuclear Decoupling Experiments on **2.14e**

Signal Irradiated ( $\delta$ )	Signal Collapsed ( $\delta$ )
1.59-1.93 (NHCHCH <sub>2</sub> CH)	4.92 to d (J=8.8 Hz) (NHCHCH <sub>2</sub> ) 4.64 to m (NHCHCH <sub>2</sub> ) 0.87 to m (CH <sub>2</sub> CH(Me) <sub>2</sub> )
2.10 (NHCHCH)	4.46 to d (J=8.3 Hz) (NHCHCH) 0.87 to m (CHCH(Me) <sub>2</sub> )
2.29 (NHCHCH)	4.64 to m (NHCHCH) 0.87 to m (CHCH(Me) <sub>2</sub> )
4.46 (NHCHCH)	2.10 to m (NHCHCH) 7.55 to s(br) (NHCHCH)
4.64 (NHCHCH <sub>2</sub> and NHCHCH)	2.29 to m (NHCHCH) 1.59-1.93 to m (NHCHCH <sub>2</sub> ) 7.99 to s(br) (NH) 6.61 to s(br) (NH)
4.92 (NHCHCH <sub>2</sub> )	1.59-1.93 to m (NHCHCH <sub>2</sub> ) 7.67 to s(br) (NHCHCH <sub>2</sub> )

Compound **2.14e** was purified by radial chromatography to give a colourless oil.

Attempts to initiate crystallisation were unsuccessful. Compound **2.14e** does not contain a plane of symmetry and thus magnetic non equivalence is evident in its NMR spectra.

The results of homonuclear decoupling experiments, performed to assign the complex <sup>1</sup>H NMR spectrum of **2.14e**, are displayed in Table 2.1. These enabled the assignment of the protons in the NHCHCH<sub>2</sub>CH and the NHCHCH moieties. Assignment of the CHCH<sub>2</sub>CH and the NHCHCH protons was also aided by published <sup>1</sup>H NMR data on Leu methyl ester hydrochloride and Val ethyl ester hydrochloride.<sup>2.32, 2.33</sup> The <sup>13</sup>C NMR

spectrum of **2.14e** was assigned by comparison to those of the Leu methyl ester cation<sup>2,31</sup> and the Val methyl ester cation.<sup>2,31</sup> The carbons of the epoxide group were assigned to signals at  $\delta$  54.8 and  $\delta$  54.3 by comparison to the  $^{13}\text{C}$  NMR spectra of other compounds in this series such as **2.14b** and **2.14c**.

#### COMPOUNDS **2.14f** and **2.14g**

The  $^1\text{H}$  NMR spectra of **2.14f** and **2.14g** were assigned by comparison to those of **2.14h** and **2.14i** (discussed below). A HMQC experiment was performed to assign the  $^{13}\text{C}$  NMR spectrum of **2.14g**. The correlations are presented in Table 2.2. The  $^{13}\text{C}$  NMR spectrum of **2.14f** was then assigned by comparison to that of **2.14g**.

Table 2.2. HMQC Data on **2.14g** and **2.14i**

Compound	$^1\text{H}$ NMR( $\delta$ )	$^{13}\text{C}$ NMR( $\delta$ )	Assignment
<b>2.14g</b>	3.00	40.5	<u>NHCH<sub>2</sub></u>
	2.09	35.2	<u>CH<sub>2</sub>CO<sub>2</sub></u>
	1.41	26.3	<u>CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub></u>
	1.29	30.6	<u>NHCH<sub>2</sub>CH<sub>2</sub></u>
	1.15	30.4, 30.4, 28.0	<u>NH(CH<sub>2</sub>)<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub></u>
<b>2.14i</b>	3.60	54.9	<u>CH(epoxide)</u>
	3.63	51.5	<u>OMe</u>
	3.19	39.4	<u>NHCH<sub>2</sub></u>
	2.27	34.0	<u>CH<sub>2</sub>CO<sub>2</sub></u>
	1.58	24.8	<u>CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub></u>
	1.45	29.2	<u>NHCH<sub>2</sub>CH<sub>2</sub></u>
	1.27	28.9, 28.8, 26.5	<u>NH(CH<sub>2</sub>)<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub></u>

#### COMPOUNDS **2.14h** and **2.14i**

Both **2.14h** and **2.14i** were purified by recrystallisation to give white solids. The

$^1\text{H}$  NMR spectrum of **2.14h** was assigned with the aid of homonuclear decoupling.

Irradiation of the quartet at  $\delta$  3.20 (NHCH<sub>2</sub>CH<sub>2</sub>) led to collapse of the NH triplet at  $\delta$  6.70

(to a broad singlet) and to collapse at  $\delta$  1.47 (to a triplet,  $J=6.8$  Hz), thus assigned to the  $\text{NHCH}_2\text{CH}_2$  group. Further, irradiation showed that the triplet at  $\delta$  2.28 ( $\text{CH}_2\text{CH}_2\text{CO}_2$ ) and the multiplet at  $\delta$  1.59 ( $\text{CH}_2\text{CH}_2\text{CO}_2$ ) were mutually coupled. The  $^1\text{H}$  NMR spectrum of **2.14i** was assigned by comparison to that of **2.14h**. In the  $^{13}\text{C}$  NMR spectrum of **2.14i**, the  $\text{CH}_2$  chain was assigned with the aid of a HMQC experiment (see Table 2.2). The  $^{13}\text{C}$  NMR spectrum of **2.14h** was assigned by comparison to that of **2.14i**.

#### COMPOUNDS **2.14j** and **2.14k**

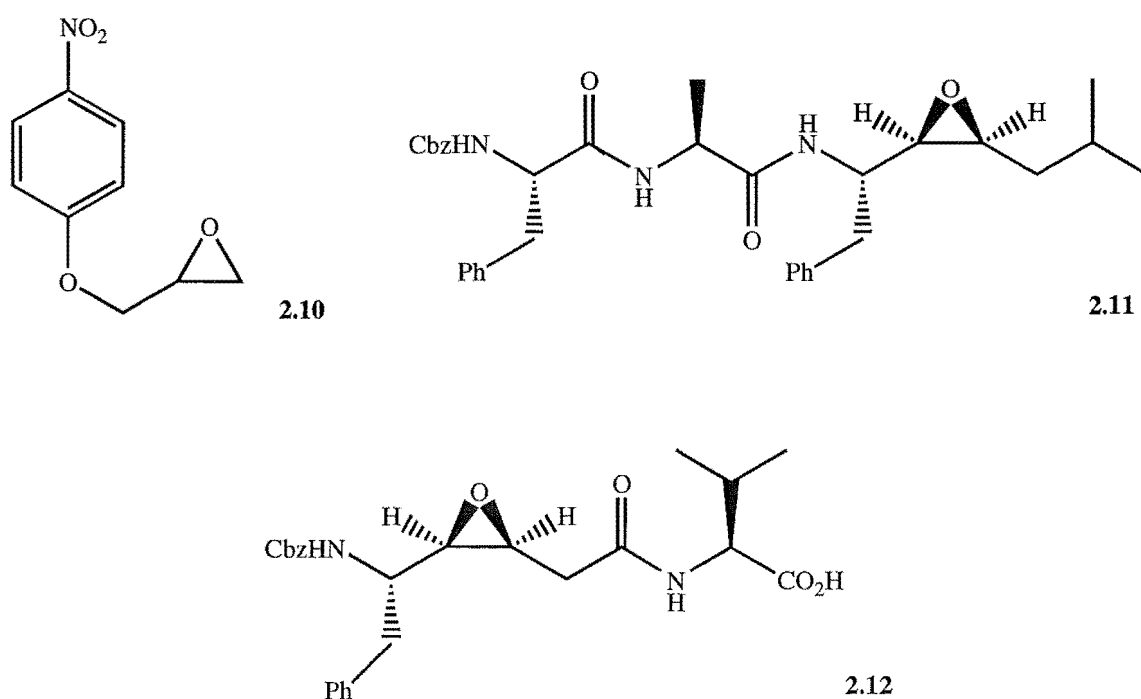
Both **2.14j** and **2.14k** were purified by recrystallisation to give white solids. The  $^1\text{H}$  NMR spectrum of **2.14k** was assigned with the aid of homonuclear decoupling. This showed the signals at  $\delta$  3.21 ( $\text{NHCH}_2$ ) and at  $\delta$  6.50 (NH) to be mutually coupled and further the signals  $\delta$  3.21 ( $\text{NHCH}_2$ ) and  $\delta$  1.46 ( $\text{NHCH}_2\text{CH}_2$ ) to be mutually coupled. The  $^1\text{H}$  NMR spectrum of **2.14j** was assigned by comparison to that of **2.14k**. The  $^{13}\text{C}$  NMR spectra of **2.14j** and **2.14k** was assigned by comparison to that of other compounds in this series such as **2.14f** and **2.14g**.

#### COMPOUND **2.20**

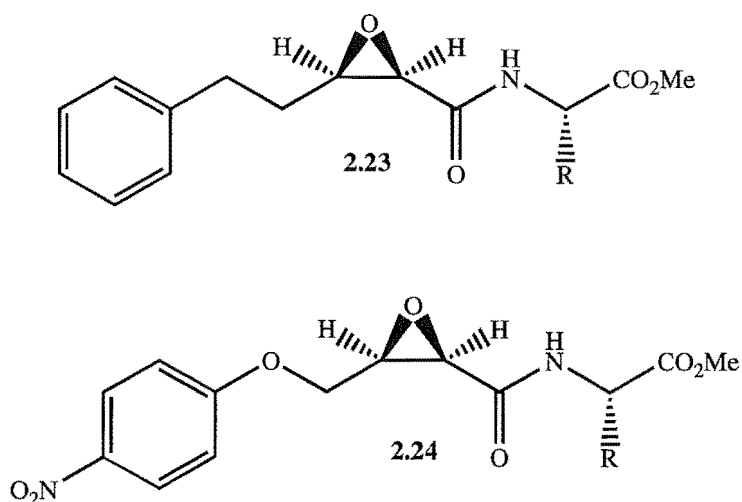
Compound **2.20** was purified by recrystallisation to give a white solid. In the  $^1\text{H}$  NMR spectrum of **2.20**, the epoxide protons appeared as an AB quartet at  $\delta$  3.75 and  $\delta$  3.68. Other  $^1\text{H}$  NMR signals were assigned by comparison to those of compound **2.14j**. Similarly  $^{13}\text{C}$  NMR signals were assigned by comparison to those of **2.14j**.

Part 2. 1,2-Epoxy-3-(*p*-nitrophenoxy)propane (ENP) Inspired Inhibitors of the HIV PR*The Synthesis of ENP Inspired Inhibitors*

As discussed in the introduction to this chapter, 1,2-epoxy-3-(*p*-nitrophenoxy)propane (ENP) (**2.10**) is an irreversible inhibitor of aspartic acid proteases, including the HIV PR.<sup>2,2, 2.19</sup> The crystal structure of **2.10** bound to the Simian Immunodeficiency Virus Protease (SIV PR) revealed the major interaction between the protease and compound **2.10** to be a covalent bond to one of the active site Asp residues.<sup>2.19</sup> Further interactions with the enzyme include the occupation of the S1 subsite by the phenyl group of **2.10** and weak interactions with the nitro group and the phenoxy oxygen of **2.10**.<sup>2.19</sup> Compound **2.10** provided a lead structure for the design of irreversible inhibitors of the HIV PR, such as compounds **2.11** and **2.12**, where salient structural features of **2.10** have been incorporated into peptide analogues.<sup>2.20, 2.21</sup> Inactivation by **2.11** and **2.12** is thought to result from alkylation of an active site Asp residue, analogous to **2.10**.<sup>2.20</sup>



This section presents the synthesis of inhibitors of the HIV PR (compounds **2.23** and **2.24**), which incorporate the important structural features of compound **2.10** into a peptide analogue. The target peptidomimetic (**2.24**) combines the epoxide, (essential for irreversible inhibition<sup>2.19, 2.20, 2.21</sup>) and the *p*-nitrophenoxy moiety of **2.10** with a suitable amino acid to enhance enzyme recognition. Compound **2.23** was considered as a model to demonstrate the feasibility of the synthesis and to probe the importance of the nitro group and phenoxy oxygen. The (*R,R*)-configuration was adopted for the epoxide of compounds **2.23** and **2.24** since studies on compounds **2.11** and **2.12** showed the (*R,S*)-form (note the priority change) of the epoxide resulted in the most potent inhibitor.<sup>2.20, 2.21</sup> Further, as discussed in the introduction to this chapter, the *cis* configuration of the epoxide was critical to the potency of compound **2.12**.<sup>2.21</sup>



The five step synthetic route to compounds **2.23** and **2.24** is shown in Scheme 2.8. Fortunately, each step was achieved readily in good yield. Step 1 involved a “Horner-Wadsworth-Emmons” (HWE)<sup>2.34</sup> reaction on the aldehydes (**2.25a** and **2.25b**) to give the olefins (**2.26a** and **2.26b**). The aldehyde (**2.25a**) was purchased from Aldrich® and **2.25b** was synthesised by a literature procedure.<sup>2.35</sup> Appropriate HWE conditions were

chosen to promote the formation of the *Z* olefin and an excess of the *Z* olefin was formed (the *E*:*Z* ratio was 1:6.4 for **2.26a** and 1:3.4 for **2.26b**). In both cases the *Z* olefin was separable by chromatography. A detailed study of the HWE reaction, presented in Chapter 3, demonstrated that an excess of the *Z* olefin was obtained for reactions with methyl diphenylphosphonoacetate, at -78 °C, in tetrahydrofuran (THF). In step 2, the olefins (**2.26a** and **2.26b**) were reduced with diisobutylaluminiumhydride (DIBAL)<sup>2.36</sup> to give the allylic alcohols (**2.27a** and **2.27b**). The reaction was performed with 2.5 equivalents of DIBAL to ensure complete conversion to the alcohol (the potential existed for incomplete reduction to give an aldehyde).

A Sharpless epoxidation<sup>2.37</sup> on **2.27a** and **2.27b** (step 3, Scheme 2.8) gave the epoxy alcohols (**2.28a** and **2.28b**). This reaction had already been demonstrated on compound **2.27a**.<sup>2.37</sup> The reaction used L-(+)-diisopropyltartrate (DIPT) to give the desired (2*S*,3*R*)-enantiomer in >95:5 excess (note that the (2*S*) stereochemistry (compound **2.27**) becomes (2*R*) in compounds **2.23**, **2.24**, and **2.29** due to a priority change at C1).

As shown in step 4 (Scheme 2.8), oxidation of the epoxy alcohols (**2.28a** and **2.28b**) was achieved by periodioic acid (H<sub>5</sub>IO<sub>6</sub>) with a catalytic amount of ruthenium trichloride hydrate (RuCl<sub>3</sub>)<sup>2.38</sup> to give the epoxy acids (**2.29a** and **2.29b**). Finally, in step 5 (Scheme 2.8), the benzotriazolyloxy-tris-(dimethylamino)phosphonium hexafluorophosphate (BOP) mediated coupling<sup>2.39</sup> of an amino acid residue with **2.29a** or **2.29b** gave the target compounds (**2.23** and **2.24**, respectively). Initially L-Leu methyl ester hydrochloride was coupled giving compounds **2.23a** and **2.24a**. Leu was chosen since it was unknown whether a P1' or P2' residue would best enhance enzyme recognition and Leu is an acceptable residue at either position.<sup>2.2</sup> Further, L-Val methyl ester hydrochloride was

coupled with the epoxy acids (**2.29a** and **2.29b**) to give the inhibitors (**2.23b** and **2.24b**).

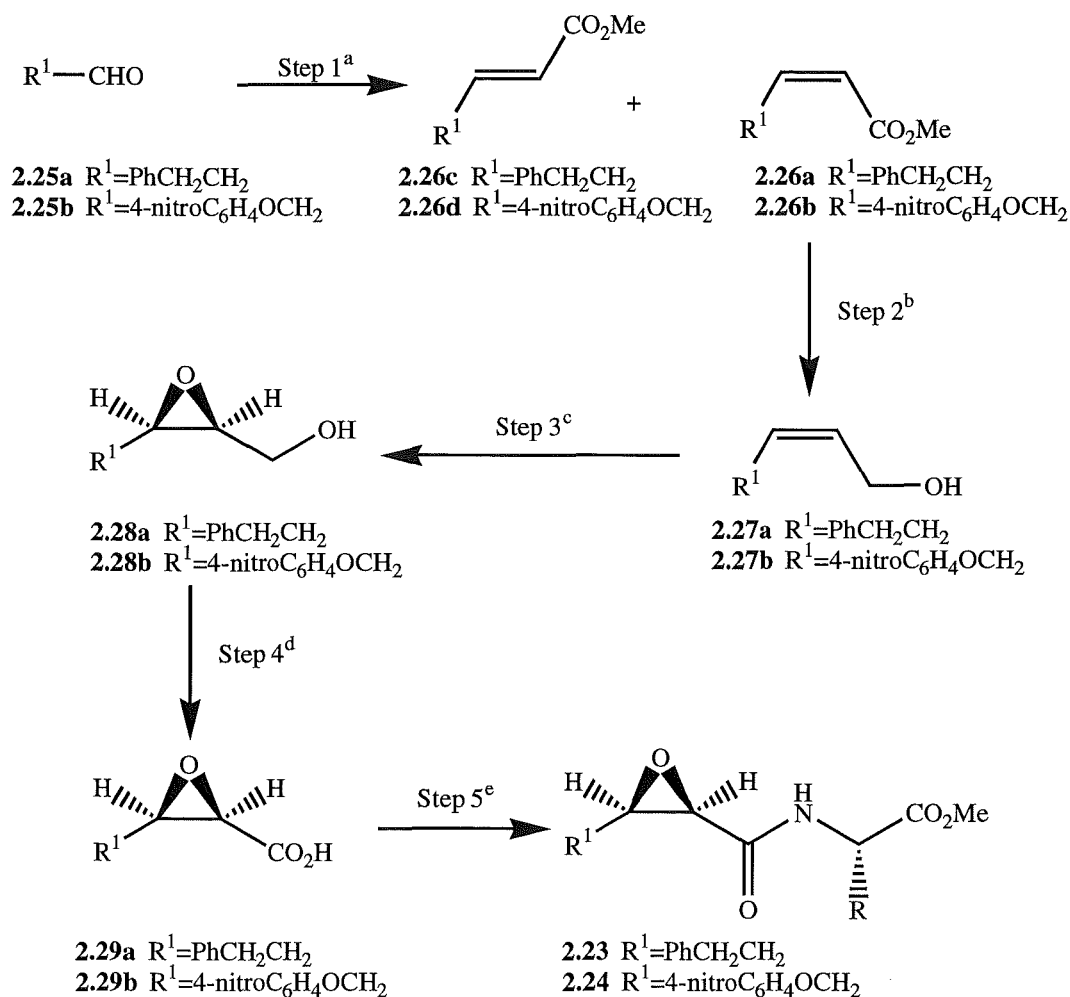
Val is a well known residue at the P2' position but not at the P1' position.<sup>2.1, 2.2</sup> A

discussion on relative activities of compounds **2.24a** and **2.24b** (and thus whether Leu or

Val is a better residue to enhance enzyme recognition for this system) is presented in part

3 of this chapter.

Scheme 2.8

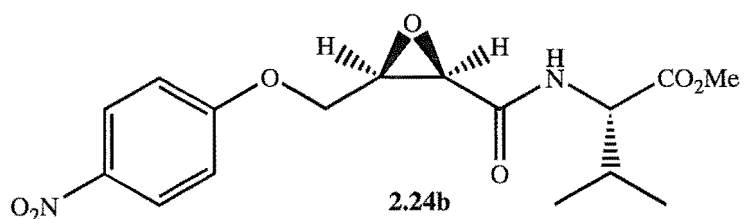
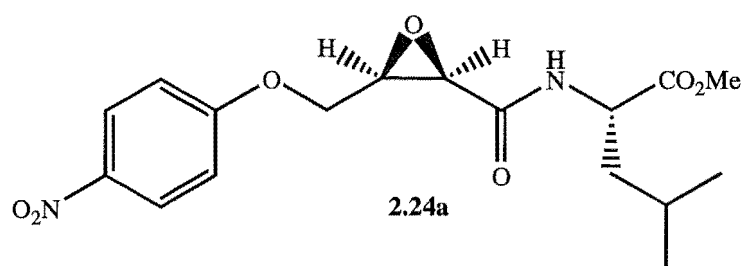
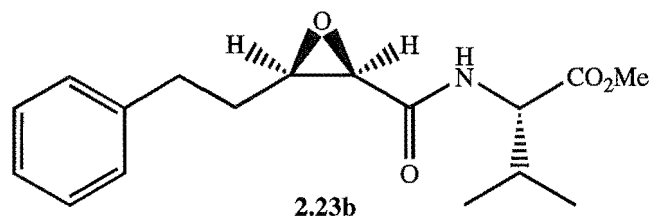
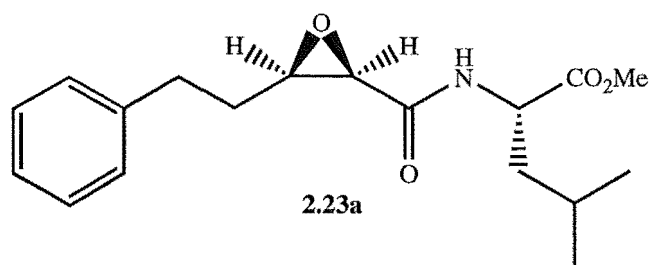


a.<sup>2.34</sup>  $n\text{-BuLi}$ ,  $(PhO)_2POCH_2CO_2Me$ , THF,  $-78^\circ C$ . b.<sup>2.36</sup> DIBAL,  $CH_2Cl_2$ ,  $-78^\circ C$ . c.<sup>2.37</sup>

$4\text{\AA}$  molecular sieves,  $Ti(OiPr)_4$ , L+ DIPT, TBHP. d.<sup>2.38</sup>  $RuCl_3$ ,  $H_5IO_6$ ,

$CCl_4/CH_3CN/H_2O$ . e.<sup>2.39</sup>  $ClH_3NCHRCO_2Me$ , BOP,  $Et_3N$ ,  $CH_2Cl_2$

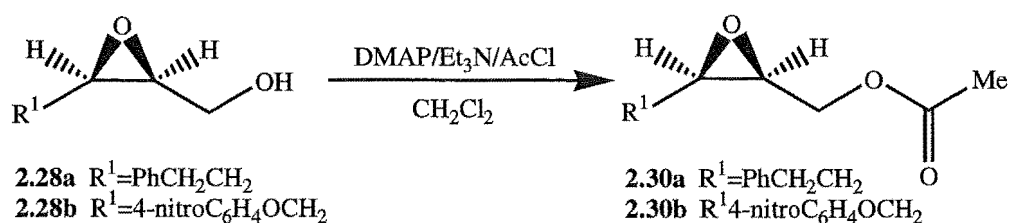




Analysis, by  $^1\text{H}$  NMR, of their crude reaction mixtures revealed that compounds **2.23a**, **2.23b**, **2.24a**, and **2.24b** were each obtained as single isomers. This demonstrates the stereoselectivity of the Sharpless epoxidation (step 3, Scheme 2.8). Further evidence for this was obtained by the determination of the enantiomeric excess of the reaction. This was achieved, as described by Sharpless *et al.*, by conversion of the epoxides (**2.28a** and **2.28b**) to the corresponding acetates (**2.30a** and **2.30b**, Scheme 2.9) followed by shift analysis.<sup>2.40</sup> This analysis involved sequential addition of the shift reagent, europium (111) tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorate], to a solution of **2.30a**

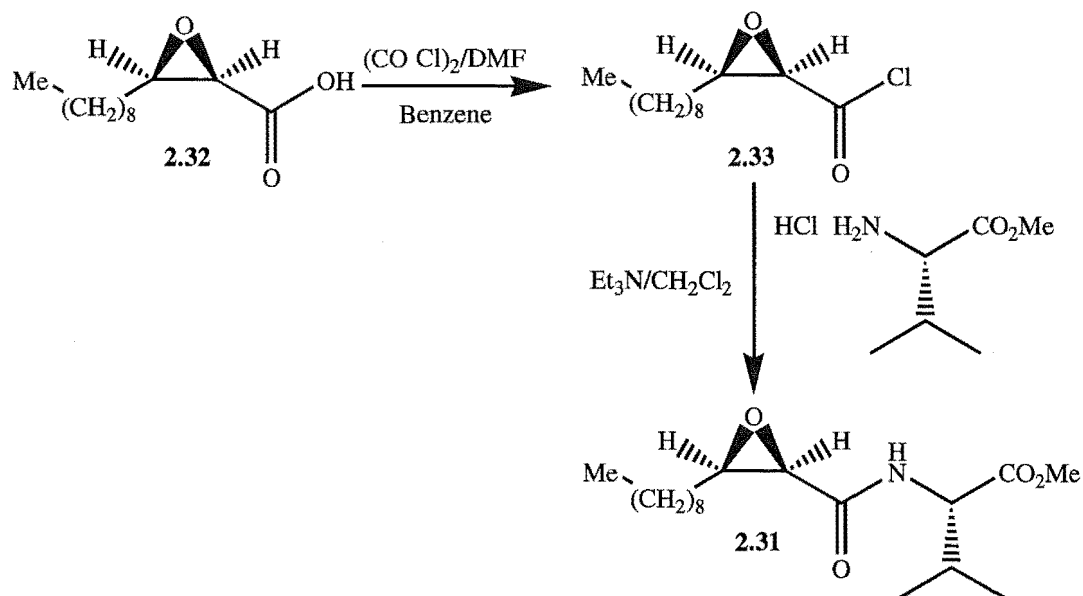
or **2.30b** and observation of the acetate Me by  $^1\text{H}$  NMR.<sup>2.40</sup> When a second resonance for the acetate Me was not observed, it was concluded that the enantiomeric excess was greater than the limit of detection by NMR (>95:5).

Scheme 2.9



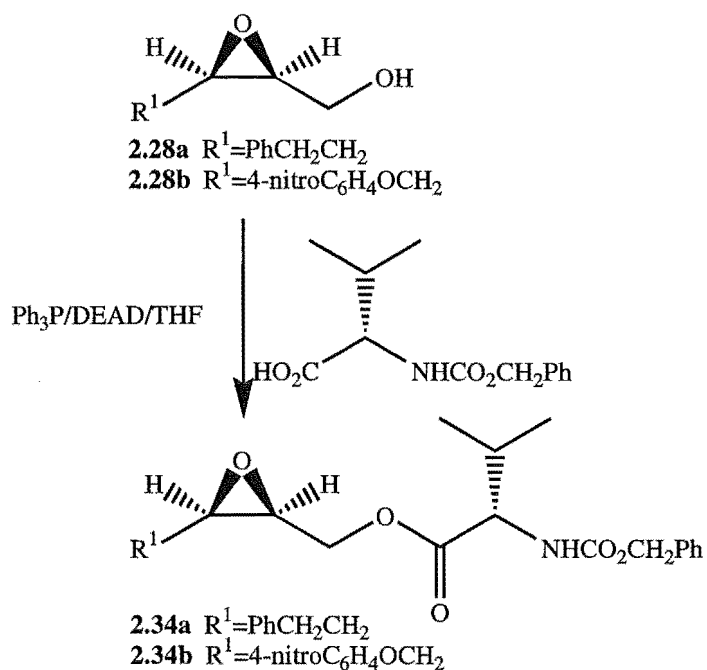
Finally, compound **2.31** (Scheme 2.10) was designed to probe the importance of the *p*-nitrophenoxy group in compounds of the type **2.24**. Compound **2.31** is an analogue of compound **2.24b** containing a simple alkyl chain place of the *p*-nitrophenoxy group. Similar compounds, such as **2.8**<sup>2.17</sup> and **2.9**,<sup>2.18</sup> also containing a nine carbon chain, have been shown to be inhibitors of the HIV PR and are discussed in the introduction to this chapter. A comparison of the *in vitro* activities of compounds **2.24b** and **2.31** is presented in part 3 of this chapter. Compound **2.31** was synthesised from compound **2.32** via the acid chloride (**2.33**) as shown in Scheme 2.10. Compound **2.32** was synthesised by a literature procedure.<sup>2.18</sup>

Scheme 2.10

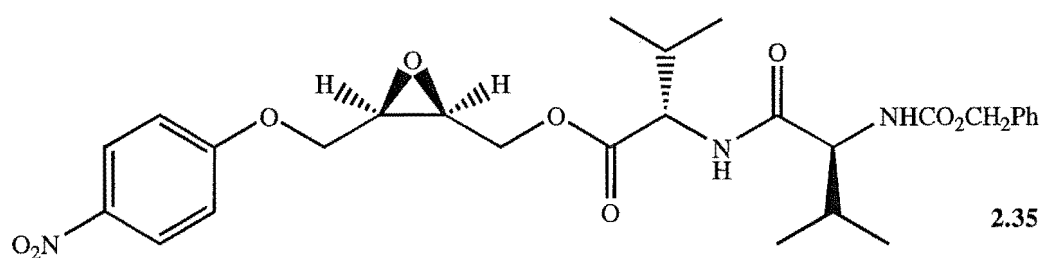


A further series of inhibitors of the HIV PR included compounds **2.34a** and **2.34b**. The diethyl azodicarboxylate (DEAD) mediated coupling<sup>2.41</sup> of the epoxy alcohols (**2.28a** and **2.28b**) with *N*-CBz-L-Val, to give compounds **2.34a** and **2.34b**, is shown in Scheme 2.11. Compounds **2.34a** and **2.34b** are peptidomimetics based on the tripeptide, Phe-Gly-Val, similar to the known inhibitor (**2.12**) (discussed in the introduction to this chapter).<sup>2.21</sup> The potential advantage of compounds **2.34a** and **2.34b**, over inhibitors of the type **2.24**, is the presence of a methylene (between the epoxide and the  $\text{CO}_2$ ). Studies on **2.12** have shown a methylene group, alpha to the epoxide group, to be essential to its activity.<sup>2.21</sup>

Scheme 2.11



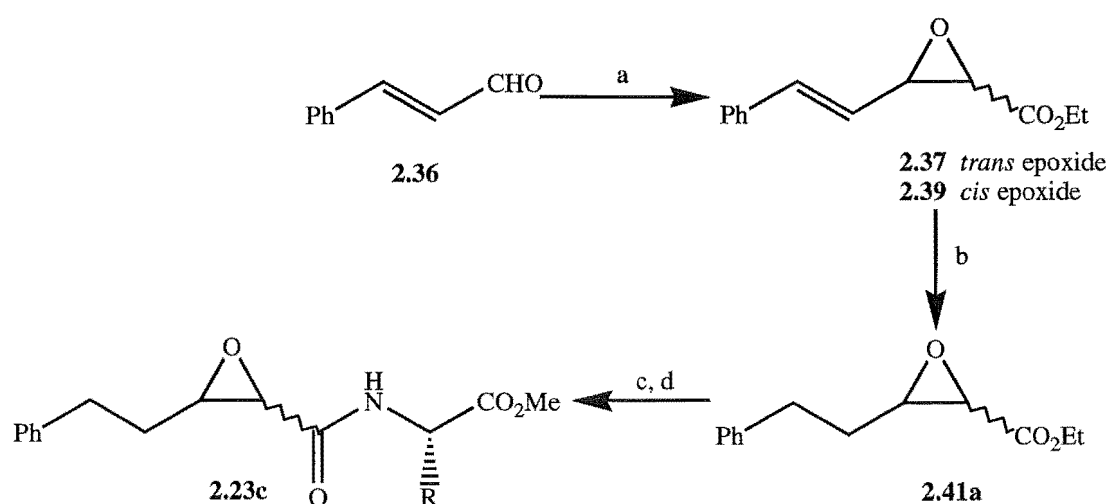
Further, the inhibitor (**2.35**) was synthesised by the DEAD mediated coupling of the epoxy alcohol (**2.28b**) with *N*-CBz-L-Val-L-Val (Scheme 2.11). Compound **2.35** is a “tetrapeptide” analogue with Val a suitable residue to enhance enzyme recognition,<sup>2.1, 2.2</sup> at the P2' and P3' positions.



## An Alternative Approach to the Synthesis of ENP Inspired Inhibitors

Initially, the synthesis of peptidomimetics of the type **2.23** was attempted via the proposed synthetic pathway shown in Scheme 2.12. However, due to problems encountered in step b (discussed below), this synthetic pathway was abandoned in favour of the efficient and stereoselective synthesis shown in Scheme 2.8.

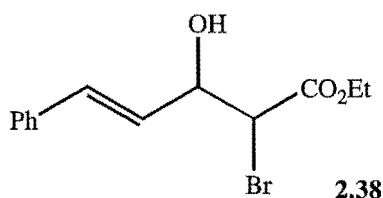
Scheme 2.12



a.<sup>2.42</sup> Lithium *bis*(trimethylsilyl)amide/ethyl bromoacetate/THF. b. H<sub>2</sub>/Pd on C<sup>2.43</sup> or rhodium on alumina.<sup>2.44</sup> c. NaOH/MeOH. d.<sup>2.39</sup> ClH<sub>3</sub>NCHRCO<sub>2</sub>Me, BOP, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>.

The investigations into step a (Scheme 2.12) gave an interesting and potentially useful synthetic result. Step a, the treatment of cinnamaldehyde (**2.36**) with ethyl bromoacetate under Darzens conditions<sup>2.42</sup> (with an excess of base), gave a mixture of two compounds—the *trans* epoxide (**2.37**) and the bromohydrin (**2.38**). Compounds **2.37** and **2.38** were separable by chromatography. Further treatment of compound **2.38** to Darzens conditions effected approximately 50% conversion to the *cis* epoxide (**2.39**) (shown by a

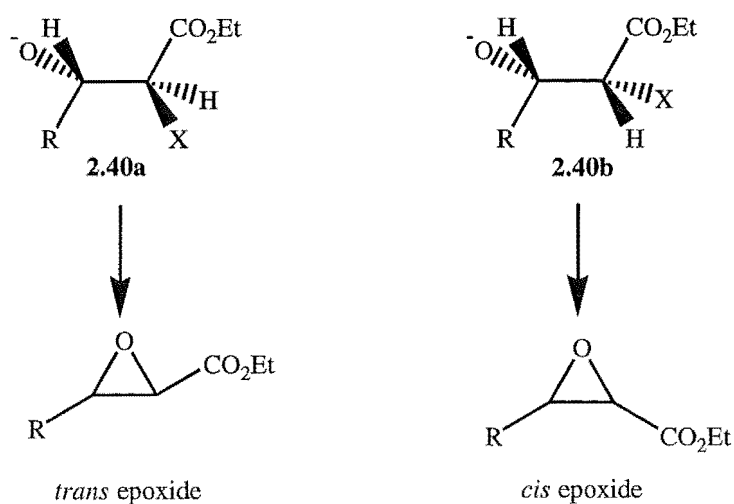
$^1\text{H}$  NMR spectrum of the crude reaction mixture). However, complete conversion to **2.39** was effected by the action of potassium *tert*-butoxide in tetrahydrofuran.<sup>2.45</sup> Therefore, the *trans* and *cis* epoxides (**2.37** and **2.39**) were obtained separately (they were not able to be separated directly by chromatography).



Although a mixture of the *trans* and *cis* epoxides is the usual outcome of the Darzen reaction, halohydrins of the type (**2.38**) have also been obtained previously.<sup>2.46</sup>

Reportedly, halohydrins are formed by protonation of the intermediate species (**2.40a** and **2.40b**, Scheme 2.13) during the aqueous work-up.<sup>2.46</sup> It has been observed that the intermediate (**2.40a**) cyclised more readily to the *trans* epoxide than did the intermediate (**2.40b**), which in some cases did not cyclise at all (thereby giving a mixture of a *trans* epoxide and a halohydrin similar to that reported above (compounds **2.37** and **2.38**)).<sup>2.46</sup>

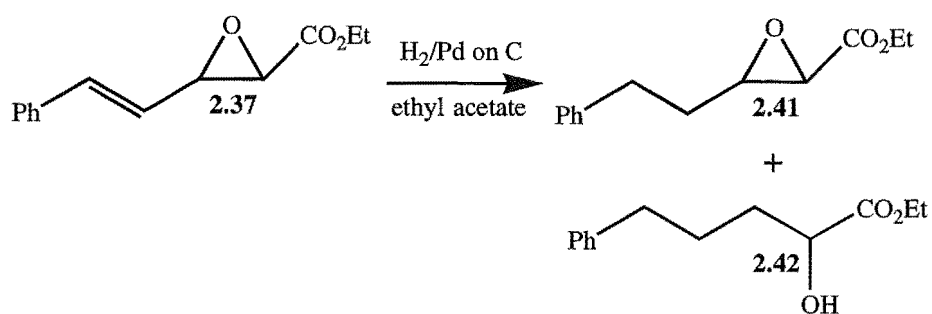
Scheme 2.13



Note: one enantiomer only of **2.40a** and **2.40b** is shown.

Finally, hydrogenation (palladium on carbon) of the *trans* epoxide (**2.37**) gave a mixture of the desired epoxide (**2.41**) and the ring opened compound (**2.42**), separable by chromatography (Scheme 2.14). Hydrogenation of the *cis* epoxide (**2.39**) gave a complex mixture. Hydrogenation over rhodium on alumina, reported to selectively saturate olefins in the presence of epoxides,<sup>2.44</sup> was also unsuccessful.

Scheme 2.14



*Structure Assignment***COMPOUNDS 2.23a and 2.23b**

The  $^1\text{H}$  NMR spectra of the crude reaction mixtures revealed that each compound (**2.23a** and **2.23b**) was obtained as a single diastereoisomer. The reaction products were purified by radial chromatography. Compounds **2.23a** and **2.23b** are previously unreported and were fully characterised. The  $^1\text{H}$  NMR spectrum of **2.23a** revealed the epoxide protons as a doublet at  $\delta$  3.53 ( $J=4.9$  Hz) (assigned to the  $\text{CHCO}$  epoxide) and a doublet of triplets at  $\delta$  3.21 ( $J=4.9, 6.4$  Hz) (assigned to the  $\text{CH}_2\text{CH}$  epoxide). In the  $^1\text{H}$  NMR spectrum of **2.23a**, the  $\text{PhCH}_2\text{CH}_2$  protons were distinguished by comparison to those of compound **2.28a**. For both compounds (**2.23a** and **2.23b**), the amino acid moiety was assigned by comparison to published  $^1\text{H}$  NMR spectra.<sup>2.32, 2.33</sup> A HMQC experiment was performed on both **2.23a** and **2.23b** to assign their  $^{13}\text{C}$  NMR spectra. The correlations are displayed in Table 2.3.

**COMPOUNDS 2.24a and 2.24b**

The  $^1\text{H}$  NMR spectra of the crude reaction mixtures revealed that each compound (**2.24a** and **2.24b**) was obtained as a single diastereoisomer. The reaction products were purified by radial chromatography. Compounds **2.24a** and **2.24b** are both previously unreported and were fully characterised. Where appropriate, the  $^1\text{H}$  NMR spectra of **2.24a** and **2.24b** were assigned by comparison to that of **2.23a** and **2.23b**. A HMQC experiment was performed to assign the  $^{13}\text{C}$  NMR spectrum of compound **2.24a** and the correlations are shown in Table 2.3. The  $^{13}\text{C}$  NMR spectrum of compound **2.24b** was assigned by comparison to those of **2.23a**, **2.23b**, and **2.24a**.



Table 2.3. HMQC Data on **2.23a**, **2.23b**, and **2.24a**

Compound	$^1\text{H}$ NMR( $\delta$ )	$^{13}\text{C}$ NMR( $\delta$ )	Assignment
<b>2.23a</b>	4.66	57.7	<u>NHCH</u>
	3.63	54.6	<u>OMe</u>
	3.53	51.7	<u>CHCO</u> (epoxide)
	3.21	49.4	<u>CH<sub>2</sub>CH</u> (epoxide)
	2.82	31.7	<u>PhCH<sub>2</sub></u>
	1.99	28.4	<u>PhCH<sub>2</sub>CH<sub>2</sub></u>
	1.55-1.70	24.2, 40.7	<u>CH<sub>2</sub>CH</u> (Me) <sub>2</sub>
	0.95	21.1, 22.3	<u>CH</u> (Me) <sub>2</sub>
<b>2.23b</b>	4.57	57.6	<u>NHCH</u>
	3.64	55.9	<u>OMe</u>
	3.55	54.6	<u>CHCO</u> (epoxide)
	3.22	51.6	<u>CH<sub>2</sub>CH</u> (epoxide)
	2.81	31.7	<u>PhCH<sub>2</sub></u>
	2.21	30.4	<u>CH</u> (Me) <sub>2</sub>
	1.95	28.5	<u>PhCH<sub>2</sub>CH<sub>2</sub></u>
<b>2.24a</b>	4.64	55.2	<u>NHCH</u>
	3.72	52.0, 52.8	<u>COCH</u> (epoxide) and <u>OMe</u>
	3.67	49.7	<u>CH<sub>2</sub>CH</u> (epoxide)
	1.52-1.71	24.3, 40.0	<u>CH<sub>2</sub>CH</u> (Me) <sub>2</sub>
	0.95	20.8, 22.3	<u>CH</u> (Me) <sub>2</sub>

COMPOUNDS **2.26a** and **2.26b**

Compounds **2.26a** and **2.26b** were fully characterised. The *Z* stereochemistry was assigned to **2.26a** and **2.26b** on the basis of  $^1\text{H}$  NMR evidence. The coupling constants of the olefin protons ( $J=11.7$  Hz) was consistent with literature data for other *Z* olefins.<sup>2.47</sup> Further, the downfield position of the CH<sub>2</sub>CH signals, due to the deshielding effect of the carbonyl group,<sup>2.48</sup> was diagnostic of the *Z* isomer. For example, the CH<sub>2</sub>CH signal of **2.26b** appeared at  $\delta$  5.25, compared to  $\delta$  4.80 for the *E* isomer (**2.26d**) (this phenomena is discussed further in Chapter 3). Homonuclear decoupling experiments, performed to assign the CH<sub>2</sub>CH signal of **2.26a**, showed that the signals at  $\delta$  6.26 (CH<sub>2</sub>CH) and at  $\delta$  2.98 (CH<sub>2</sub>CH) were mutually coupled. The presence of allylic coupling was noted in the  $^1\text{H}$  NMR spectra of both **2.26a** and **2.26b** ( $J=2.0$  and  $2.4$  Hz, respectively). The

assignment of the  $^{13}\text{C}$  NMR spectra of **2.26a** and **2.26b** was aided by comparison to those of the olefins (**3.5**), discussed in Chapter 3. A satisfactory micro analysis was obtained on compound **2.26b**.

#### COMPOUNDS **2.27a** and **2.27b**

Compounds **2.27a** and **2.27b** were purified by radial chromatography and were fully characterised. In the  $^1\text{H}$  NMR spectra of **2.27a** and **2.27b**, the assignment of the  $\text{CH}_2\text{OH}$  protons was confirmed by homonuclear decoupling experiments. For example, in the  $^1\text{H}$  NMR spectrum of **2.27b**, irradiation of the signal at  $\delta$  4.32 ( $\text{CH}_2\text{OH}$ ) lead to collapse of the  $\text{CHCH}_2\text{OH}$  signal (to a doublet at  $\delta$  5.91,  $J=11.7$  Hz) and of the OH triplet at  $\delta$  1.51 (to a singlet). A HMQC experiment, performed to assign the  $^{13}\text{C}$  NMR spectrum of **2.27b**, distinguished the  $\text{C}_6\text{H}_4\text{OCH}_2$  signal at  $\delta$  64.1 and demonstrated that the  $^{13}\text{C}$  NMR signal at  $\delta$  125.4 was due to two carbons (one of the olefins and the  $\text{NO}_2\text{CCH}$  carbon). The  $^{13}\text{C}$  NMR spectrum of **2.27a** was assigned by comparison to those of **2.27b** and **2.26a**.

#### COMPOUNDS **2.28a** and **2.28b**

Compounds **2.28a** and **2.28b**, purified by radial chromatography, were fully characterised. All signals in the  $^1\text{H}$  NMR spectrum of **2.28a** appeared as multiplets and, therefore, the direct assignment of signals on the basis of their coupling was not feasible. Instead, the spectrum was assigned on the basis of homonuclear decouplings experiments (see Table 2.4). Interestingly, the  $\text{PhCH}_2\text{CH}_2$  protons appeared as four separate multiplets between  $\delta$  1.81 and  $\delta$  2.88. Homonuclear decoupling experiments were also employed to assign the  $^1\text{H}$  NMR spectrum of **2.28b** (see Table 2.4). The  $\text{C}_6\text{H}_4\text{OCH}_2$

signal appeared as two separate doublets of doublets, as did the  $\text{CH}_2\text{OH}$  signal after irradiation of either the OH triplet or the  $\text{CHCH}_2\text{OH}$  signal (see Table 2.4). To assign the  $^{13}\text{C}$  NMR spectrum of **2.28a**, a distortionless enhancement by polarisation transfer (DEPT) experiment was performed to distinguish the epoxide carbons at  $\delta$  56.6 and  $\delta$  56.1 from the  $\text{CH}_2\text{OH}$  carbon at  $\delta$  60.0 (the olefins were used as the reference carbons). The  $^{13}\text{C}$  NMR spectrum of **2.28b** was assigned by comparison to those of **2.28a** and **2.27b**.

**Table 2.4. Homonuclear Decoupling Experiments on 2.28a and 2.28b**

Compound	Signal Irradiated ( $\delta$ )	Signal Collapsed ( $\delta$ ) (coupling constant (Hz))
<b>2.28a</b>	3.57 ( $\text{CH}_2\text{OH}$ )	1.34 to s (OH) and 3.11 (epoxide)
	3.11 (epoxide)	3.57 ( $\text{CH}_2\text{OH}$ ) and 1.81, 2.01 ( $\text{PhCH}_2\text{CH}_2$ )
	2.88 ( $\text{PhCH}_2$ )	1.81, 2.01 ( $\text{PhCH}_2\text{CH}_2$ ) and 2.73 ( $\text{PhCH}_2$ )
	2.73 ( $\text{PhCH}_2$ )	1.81, 2.01 ( $\text{PhCH}_2\text{CH}_2$ ) and 2.88 ( $\text{PhCH}_2$ )
	2.01 ( $\text{PhCH}_2\text{CH}_2$ )	1.81 ( $\text{PhCH}_2\text{CH}_2$ ) and 2.73, 2.88 ( $\text{PhCH}_2$ )
	1.81 ( $\text{PhCH}_2\text{CH}_2$ )	2.01 ( $\text{PhCH}_2\text{CH}_2$ ) and 2.73, 2.88 ( $\text{PhCH}_2$ )
	1.34 (OH)	3.57 to d ( $J=5.9$ ) ( $\text{CH}_2\text{OH}$ )
<b>2.28b</b>	3.98 ( $\text{CH}_2\text{OH}$ )	1.77 (OH), 3.34 ( $\text{CHCH}_2\text{OH}$ ), and 3.87 ( $\text{CH}_2\text{OH}$ )
	3.87 ( $\text{CH}_2\text{OH}$ )	1.77 (OH), 3.34 to t ( $J=4.9$ ) ( $\text{CHCH}_2\text{OH}$ ), and 3.98 ( $\text{CH}_2\text{OH}$ )
	3.47 ( $\text{C}_6\text{H}_4\text{OCH}_2\text{CH}$ )	3.34 ( $\text{CHCH}_2\text{OH}$ ) and 4.28, 4.37 to ABq ( $J=11.7$ ) ( $\text{C}_6\text{H}_4\text{OCH}_2$ )
	3.34 ( $\text{CHCH}_2\text{OH}$ )	3.47 ( $\text{C}_6\text{H}_4\text{OCH}_2\text{CH}$ ), 3.98 to dd ( $J=5.4$ , 12.7), and 3.87 to dd ( $J=5.9$ , 12.7) ( $\text{CH}_2\text{OH}$ )
	1.77 (OH)	3.98 to dd ( $J=3.9$ , 12.7) and 3.87 to dd ( $J=4.9$ , 12.7) ( $\text{CH}_2\text{OH}$ )

Note: unless otherwise stated, signals did not collapse to a recognisable shape.

#### COMPOUNDS **2.29a** and **2.29b**

The  $^1\text{H}$  NMR spectra of **2.29a** and **2.29b** showed that the oxidation was complete—the signals due to the  $\text{CH}_2\text{OH}$  protons had disappeared and no signals had appeared in the

aldehyde region of the spectrum. The NMR spectra of **2.29a** and **2.29b** were assigned by comparison to those of **2.28a** and **2.28b**.

### COMPOUND **2.31**

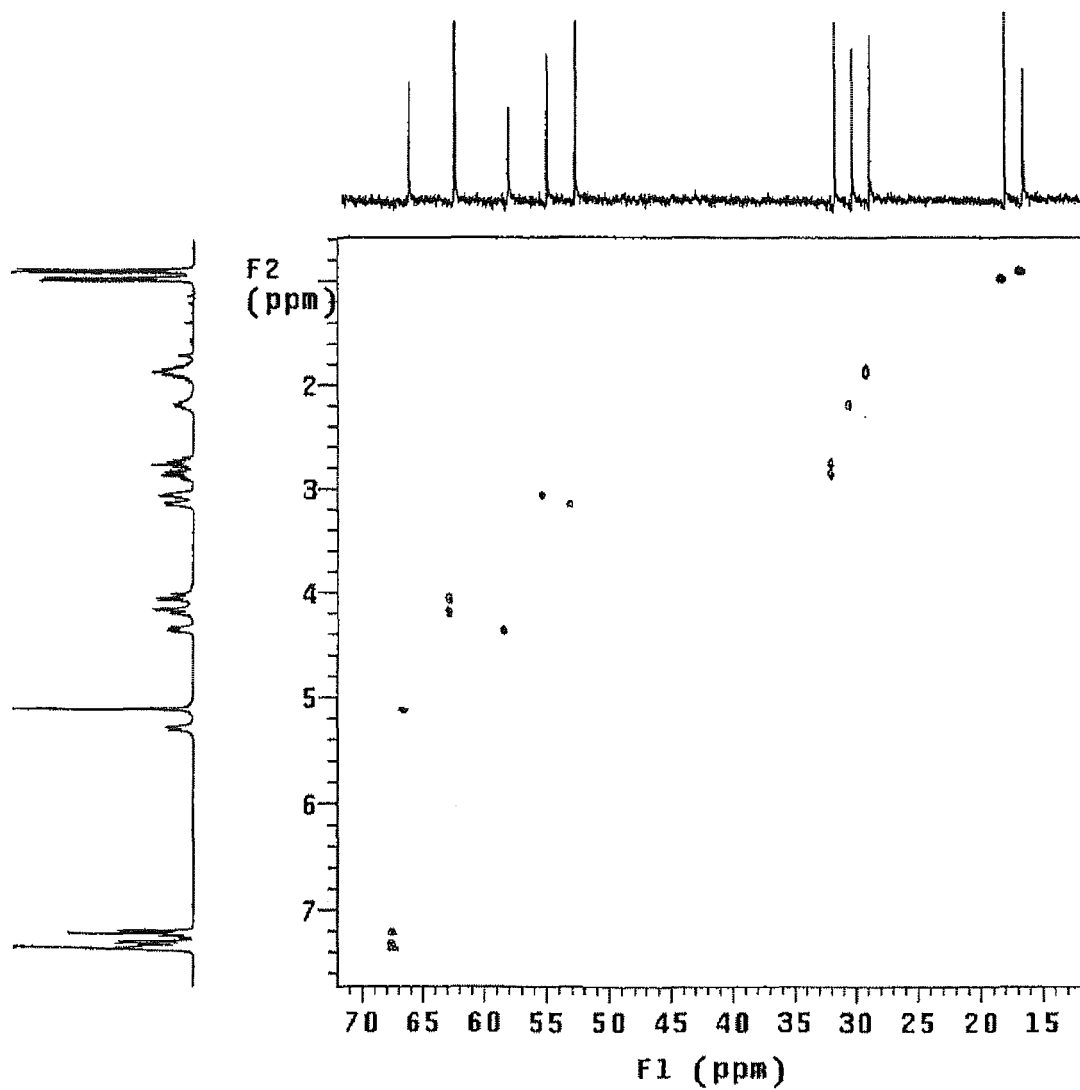
Compound **2.31**, purified by radial chromatography, was fully characterised. NMR spectra were assigned by comparison to similar compounds such as **2.23b** as appropriate.

### COMPOUNDS **2.34a**, **2.34b** and **2.35**

The  $^1\text{H}$  NMR spectra of the crude reaction mixtures revealed that each compound (**2.34a**, **2.34b**, and **2.35**) was obtained as a single diastereoisomer. The reaction products were purified by radial chromatography and were fully characterised. In the  $^1\text{H}$  NMR spectrum of **2.34a**, the  $\text{CH}_2\text{O}$  signal appeared as two separate doublets of doublets at  $\delta$  4.19 and  $\delta$  4.05. Irradiation of these signals collapsed the epoxide ( $\text{CHCH}_2\text{O}$ ) signal at  $\delta$  3.14 (to a doublet,  $J=3.6$  Hz). Further homonuclear decoupling experiments showed that the signals at  $\delta$  3.06 ( $(\text{CH}_2\text{CH}_2\text{CH})$ ) and at  $\delta$  1.87 ( $(\text{CH}_2\text{CH}_2\text{CH})$ ) were mutually coupled. In the  $^1\text{H}$  NMR spectra of **2.34b** and of **2.35**, both  $\text{OCH}_2$  signals appeared as a single multiplet, shown by homonuclear decoupling experiments to be coupled to the epoxide signals (which appeared at  $\delta$  3.36 and  $\delta$  3.45 for both compounds). A HMQC experiment, performed to assign the  $^{13}\text{C}$  NMR spectrum of **2.34a**, is shown in Figure 2.6. This distinguished the  $\text{PhCH}_2\text{CH}_2$  signals (at  $\delta$  32.1 and  $\delta$  29.7) from each other and from the  $\text{CH}(\text{Me})_2$  signal at  $\delta$  30.7 and further enabled the assignment of the epoxide carbons ( $\delta$  55.3 and  $\delta$  53.1) and the  $\text{CHCH}(\text{Me})_2$  carbon ( $\delta$  58.5). A HMQC experiment was also performed to assign the  $^{13}\text{C}$  NMR spectrum of **2.35**, particularly to distinguish the epoxide carbons ( $\delta$  52.3 and  $\delta$  53.3) and both the  $\text{CHCH}(\text{Me})_2$  carbons ( $\delta$  56.7 and

$\delta$  59.9). The  $^{13}\text{C}$  NMR spectrum of **2.34b** was assigned by comparison to those of **2.34a** and **2.35**.

Figure 2.6. The HMQC of Compound **2.34a**



COMPOUNDS **2.37** and **2.39**

The configuration of the epoxide was assigned on the basis of the coupling constants of the epoxide protons in the  $^1\text{H}$  NMR spectra of compounds **2.37** and **2.39**. For **2.37**, the epoxide protons appeared as a doublet at  $\delta$  3.49 ( $J=2.0$  Hz) and a multiplet at  $\delta$  3.76 (which collapsed to a doublet ( $J=2.1$  Hz) on irradiation at  $\delta$  5.88 ( $\text{PhCHCH}$ ). These coupling constants are consistent with *trans* epoxides.<sup>2,47</sup> Further, nuclear Overhauser enhancement (nOe) experiments were performed on compound **2.37** to confirm the *trans* assignment of the epoxide. Irradiation at  $\delta$  3.49 ( $\text{CHCO}$ ) gave enhancement at  $\delta$  5.88 ( $\text{PhCHCH}$ ) and *vice versa*. These protons are close (in space) in the *trans* epoxide (**2.37**) but not in the *cis* epoxide (**2.39**) (see Figure 2.7). As expected for an *trans* epoxide, a nOe was not obtained between the epoxide protons of compound **2.37**. For compound **2.39** the *cis* configuration was assigned to the epoxide on the basis of the  $^1\text{H}$  NMR coupling constants of the epoxide protons. The epoxide protons appeared as a multiplet at  $\delta$  3.78 which collapsed to a doublet at  $\delta$  3.80 ( $J=4.4$  Hz) and a doublet at  $\delta$  3.76 ( $J=4.4$  Hz), after irradiation at  $\delta$  6.15 ( $\text{PhCHCH}$ ). These coupling constants are consistent with *cis* epoxides.<sup>2,47</sup> The  $^{13}\text{C}$  NMR spectra of compounds **2.37** and **2.39** both required a HMQC experiment to distinguish the olefin carbons from the aromatic carbons and the epoxide carbons from the  $\text{OCH}_2$  carbon. The correlations are shown in Table 2.5.

Figure 2.7. Protons (in bold) which are close (in space) in **2.37** but not in **2.39**.

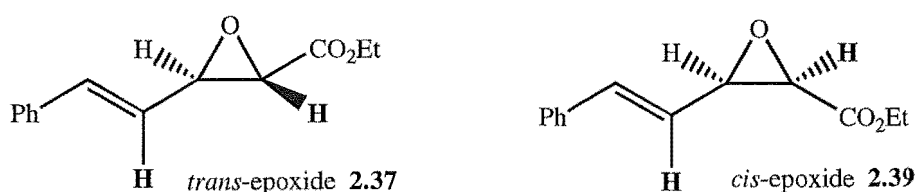


Table 2.5. HMQC Data on **2.37** and **2.39**

Compound	$^1\text{H}$ NMR( $\delta$ )	$^{13}\text{C}$ NMR( $\delta$ )	Assignment
<b>2.37</b>	6.87	136.4	PhCH
	5.88	129.9	PhCHCH
	4.28	61.8	OCH <sub>2</sub>
	3.76	58.3	CHCHCO
	3.49	55.1	CHCO
<b>2.39</b>	6.91	137.9	PhCH
	6.15	121.5	PhCHCH
	4.29	61.6	OCH <sub>2</sub>
	3.78	54.6, 57.9	CHCH(epoxides)

COMPOUND **2.38**

The molecular formula determined by mass spectrometry was consistent with the proposed structure, and the infrared spectrum indicated the presence of an OH group (assigned to a broad peak at  $3459\text{ cm}^{-1}$ ).<sup>2,47</sup> To assign the  $^1\text{H}$  NMR spectrum of **2.38**, homonuclear decoupling experiments were performed. The signals at  $\delta$  6.18 (PhCHCH) and at  $\delta$  4.70 (later assigned CHOH) were found to be mutually coupled and further the signals at  $\delta$  4.70 and at  $\delta$  4.35 (later assigned CHBr) were found to be mutually coupled. A HMQC experiment correlated the  $^1\text{H}$  NMR signal at  $\delta$  4.70 to the  $^{13}\text{C}$  NMR signal at  $\delta$  72.5 (assigned CHOH on the basis of the chemical shift of the carbon<sup>2,47</sup>) and the  $^1\text{H}$  NMR signal at  $\delta$  4.35 to the  $^{13}\text{C}$  NMR signal at  $\delta$  51.5 (assigned CHBr on the basis of the chemical shift of the carbon<sup>2,47</sup>). NMR data indicated the presence of a single diastereoisomer, which was confirmed since **2.38** was converted stereoselectively to the *cis* epoxide (**2.39**) (see Scheme 2.13 for a general scheme).

COMPOUND **2.41**

The NMR spectra of compound **2.41** was assigned by comparison to similar compounds such as **2.37** and **2.29a**.

## COMPOUND 2.42

The structure (2.42) was determined by examination of NMR data. A mass spectrum was consistent with the proposed structure. In the  $^1\text{H}$  NMR spectrum of 2.42, the four proton multiplet at  $\delta$  1.66-1.87 ( $(\text{CH}_2)_2\text{CHOH}$ ) was shown by homonuclear decoupling to be mutually coupled to the  $\text{CHOH}$  proton at  $\delta$  4.19, which appeared as a triplet ( $J=4.9$  Hz). To assign the  $^{13}\text{C}$  NMR spectrum of compound 2.42, a HMQC experiment was performed to distinguish the  $\text{PhCH}_2$  carbon at  $\delta$  35.5 from the  $\text{PhCH}_2(\text{CH}_2)_2$  carbons at  $\delta$  33.9 and at  $\delta$  26.5. The  $\text{CHOH}$  carbon was assigned on the basis of its chemical shift to the signal at  $\delta$  70.3.



Part 3. *In Vitro* Testing against the HIV-1 PR

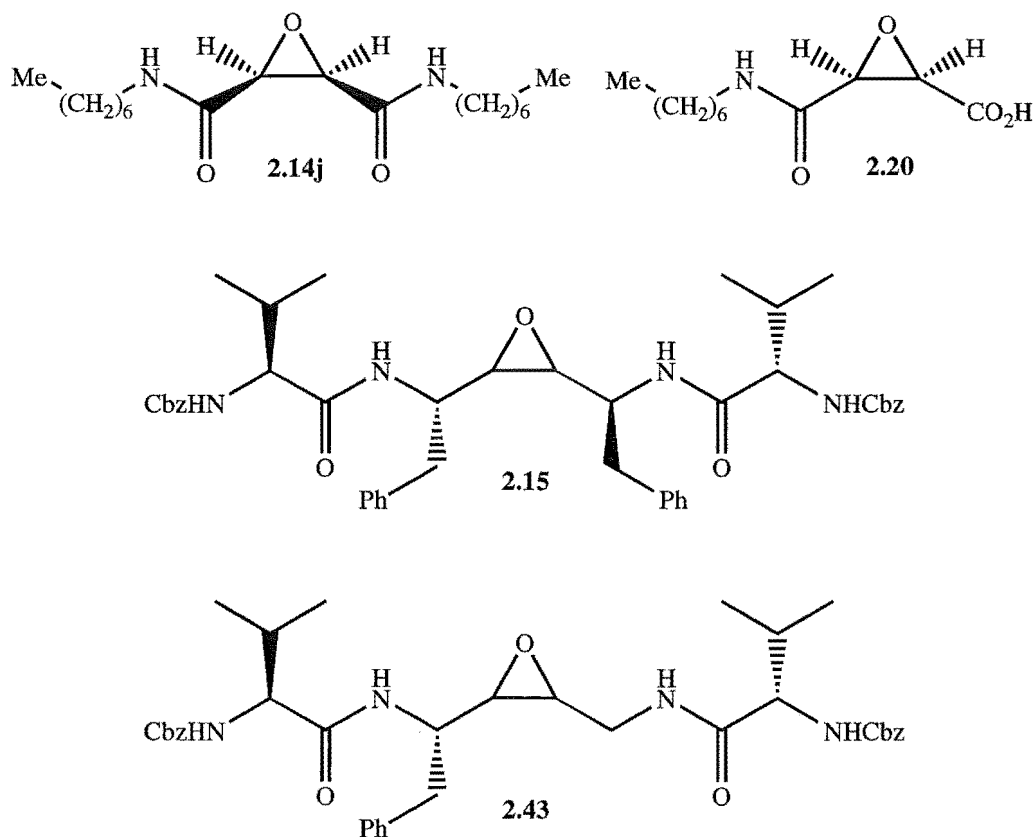
*In Vitro* Testing of compounds **2.14b-g**, **2.14j**, **2.20**, **2.23a**, **2.24a**, **2.24b**, **2.29b**, **2.31**, **2.34b**, and **2.35** against the HIV-1 PR was performed by Drs. D. Fairlie and D. Bergman of the Centre for Drug Design and Development, University of Queensland, according to a published procedure.<sup>2,49</sup> The results are displayed in Table 2.6.

Table 2.6 Inhibition Data for Epoxide-Based Compounds

Compound	IC <sub>50</sub>	K <sub>i</sub>	% Inhibition (at 200 µM)
<b>2.14b</b> , <b>2.14c</b> , and <b>2.14e</b>	—	—	inactive
<b>2.14f</b> , <b>2.14g</b> , and <b>2.14j</b>	—	—	inactive
<b>2.20</b>	—	—	9.8
<b>2.23a</b>	—	—	19.1
<b>2.24a</b>	—	—	30.2
<b>2.24b</b>	710 µM	210 µM	—
<b>2.29b</b>	—	—	15.1
<b>2.31</b>	—	—	inactive
<b>2.34b</b>	800 nM	200 nM	—
<b>2.35</b>	6 µM	1.7 µM	—

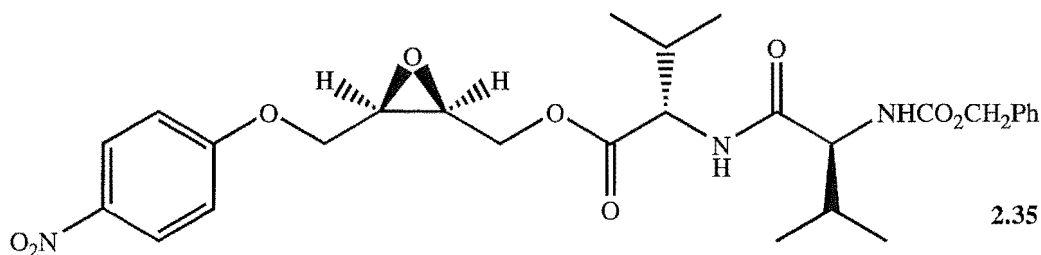
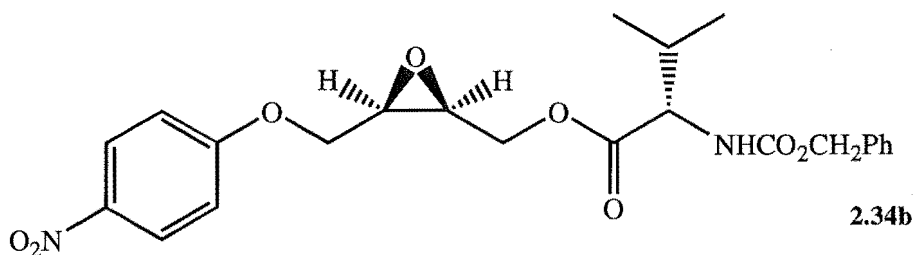
As shown in Table 2.6, the peptide-based pseudosymmetrical epoxides (**2.14b**, **2.14c**, and **2.14e**, Part 1) were inactive (at 200 µM). Further, the symmetrical epoxides (**2.14f**, **2.14g**, and **2.14j**, Part 1) were also inactive. Interestingly, however, the non-symmetrical

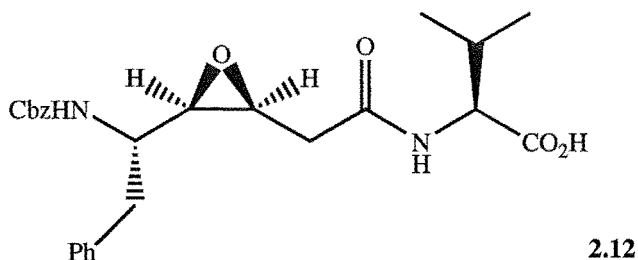
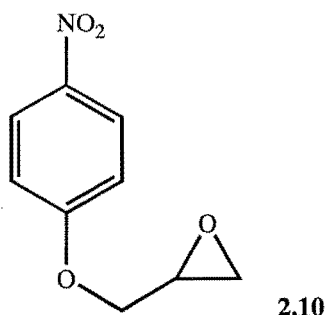
epoxide (**2.20**, Part 1) showed modest activity (9.8% at 200  $\mu$ M). Compound **2.20** was designed as an analogue of compound **2.14j** to probe the importance of the symmetrical nature of such compounds. A comparison of the *in vitro* results for **2.20** and **2.14j** (Table 2.6) suggests that the symmetrical nature of compound **2.14j** may contribute to its inactivity. This suggestion is supported by literature on the pseudosymmetrical epoxide (**2.15**).<sup>2,23</sup> Compound **2.15** is a reversible inhibitor of the HIV PR ( $K_i=75$  nM), whereas non-symmetrical analogues of **2.15**, such as compound **2.43**, exhibited irreversible inactivation of the enzyme ( $K_i=0.1$  nM).<sup>2,23</sup> The authors suggested that the rigidity of the epoxide (together with Phe at the P1' position) did not allow **2.15** to adopt the conformation required for irreversible inactivation.



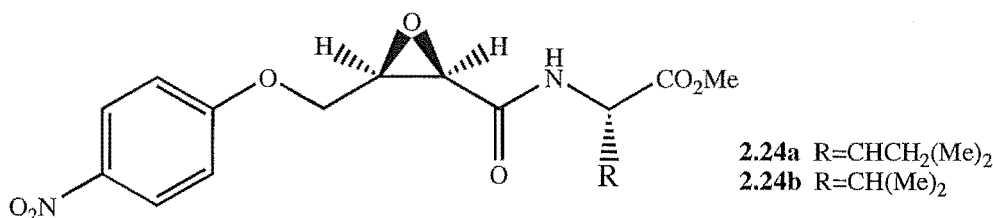
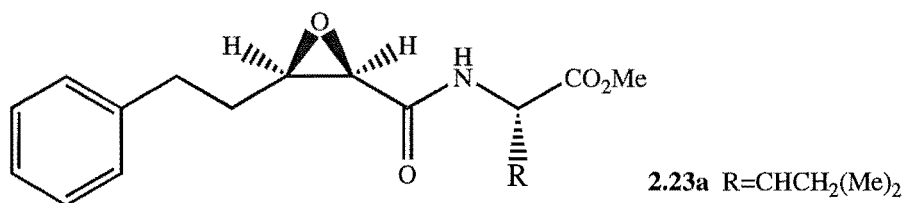
The ENP inspired peptidomimetics (**2.23a**, **2.24a**, **2.24b**, **2.29b**, **2.34b**, **2.35**, Part 2) were tested against the HIV-1 PR and were found to be active (Table 2.6). Notably, compound

**2.34b** stood out with a  $K_i$  value of 200 nM. Such activity is superior to the epoxide-based inhibitors **2.10** ( $K_i=9.9$  mM),<sup>2,19</sup> **2.11** ( $K_i=20$   $\mu$ M),<sup>2,20</sup> and **2.12** (1.32  $\mu$ M)<sup>2,21</sup> which served as lead structures (see Part 2 of this chapter). Exhaustive dialysis failed to restore enzyme activity, indicating that inactivation by compound **2.34b** was irreversible, as predicted. Further, the addition of a reversible competitive inhibitor protected the enzyme from inactivation by **2.34b**, indicating that compound **2.34b** reacts with an active site residue. It is noteworthy that compounds **2.34b** and **2.35** were considerably more active than compounds of the type **2.24**. This is perhaps due to presence of the methylene (between the epoxide and the CO<sub>2</sub>) in compounds **2.34b** and **2.35**. Studies on the known inhibitor (**2.12**) have shown a methylene group, alpha to the epoxide group, to be essential to its activity.<sup>2,21</sup> Note further, that in compounds **2.34b** and **2.35** the amino acid sequence is inverted (at the Val residue of compound **2.34b** and at the Val-Val moiety of **2.35**). Unexpectedly, the “tetrapeptide” (**2.35**), which contains Val at the P3' position, is less active than the “tripeptide” (**2.34b**).

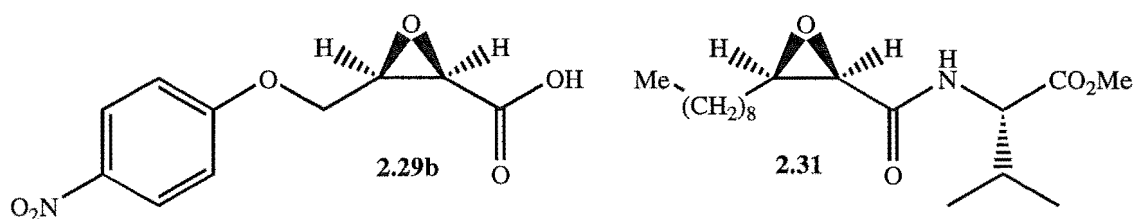




Compounds **2.23a** (19.1% inhibition at 200  $\mu$ M), **2.24a** (30.2% inhibition at 200  $\mu$ M), and **2.24b** ( $K_i$ =210  $\mu$ M) displayed moderate activity. They were, in fact, considerably more active than ENP (**2.10**) ( $K_i$ =9.9 mM),<sup>2,19</sup> which served as a lead structure (see Part 2 of this chapter). A comparison of the activities of compounds **2.24a** and **2.24b** demonstrates that Val, rather than Leu, is the better residue to enhance enzyme recognition in this system. Since Val is well known at the P2' position but not at P1',<sup>2.1, 2.2</sup> this result suggests the amino acid moiety may bind in the S2' subsite of the enzyme. Further, a comparison of the activities of compounds **2.23a** and **2.24a** demonstrates that a *p*-nitrophenoxy moiety rather than a phenylmethylene group leads to a superior inhibitor (in this system). This suggests interactions between the nitro group and phenoxy oxygen of **2.24a** and the enzyme. The crystal structure of the known inhibitor (**2.10**) bound to the SIV PR revealed weak interactions between the nitro group and phenoxy oxygen of **2.10** and the enzyme.<sup>2,19</sup>



A comparison of the activity of the parent acid (**2.29b**) with **2.24a** or **2.24b** suggests that, as expected, the addition of an amino acid residue increases inhibitor activity, presumably by enhancing enzyme recognition and binding affinity. Further, the superior activity of **2.24a** and **2.24b**, over **2.29b**, indicates that enzymatic hydrolysis of the amide bond alpha to the epoxide is unlikely to occur to a significant extent (enzymatic hydrolysis of **2.24a/2.24b** would give **2.29b** and therefore **2.24a/2.24b** would give a similar *in vitro* result to **2.29b**).



Finally, compound **2.31**, designed to probe the importance of the *p*-nitrophenoxy group in compounds of the type **2.24**, proved to be inactive at 200  $\mu\text{M}$ . Compound **2.31** is an analogue of compound **2.24b** containing a simple alkyl chain in place of the *p*-nitrophenoxy group. This result demonstrates that the *p*-nitrophenoxy group is critical to the activity of **2.24b**.

## Experimental

### General Procedure

Melting points (m.p.) were taken on a Reichert hot-stage microscope and are uncorrected. Nuclear Magnetic Resonance (NMR) spectra were recorded on either a Varian CFT300 or a XL300 spectrometer. Mass spectra (MS) were obtained on a Kratos MS80RFA spectrometer. Infrared (IR) spectra were recorded on a Shimadzu FTIR-8201PC spectrophotometer. Optical rotations were measured on a JASCO J-20C recording spectropolarimeter and  $[\alpha]_D$  values are given in units of  $10^{-1}$  degrees  $\text{cm}^2 \text{g}^{-1}$ , with the concentration (c) given in units of  $\text{mg cm}^{-3}$ . Radial chromatography was performed on a Chromatotron (Harrison and Harrison) using Merck type 60 PF254 silica gel. The amino acids (glycine ethyl ester hydrochloride, L-leucine methyl ester hydrochloride, L-phenylalanine methyl ester hydrochloride, glycylglycine ethyl ester hydrochloride, *N*-Boc-L-leucine, L-valine benzyl ester hydrochloride, L-valine methyl ester hydrochloride, *N*-CBz-L-valine, and *N*-CBz-L-valinyl-L-valine), the amines (**2.19**,  $n=6/7$ ), and the aldehyde (**2.25a**) were commercially available.

### Preparation<sup>2,24</sup> of (2*S*,3*R*)-*N,N*-Ethylglyciny-2,3-epoxybutandiamide (**2.14a**)

To a stirring solution of 100 mg (0.76 mmol) of **2.16** (synthesised by a literature procedure<sup>2,25</sup>) in dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) (5.0 ml), at 0 °C, were added two equivalents (1.5 mmol) of each glycine ethyl ester hydrochloride (209 mg), triethylamine ( $\text{Et}_3\text{N}$ ) (152 mg), dicyclohexylcarbodiimide (DCC) (309 mg), and 1-hydroxybenzotriazole (HOBT) (203 mg). The resultant solution was stirred at room temperature (r.t.) overnight

after which the solvent was evaporated under reduced pressure. Ethyl acetate was then added to the residue to precipitate the dicyclohexylurea (DCU) byproduct, which was removed by filtration. The filtrate was washed (3x) with saturated sodium bicarbonate ( $\text{NaHCO}_3$ ), dried with magnesium sulfate ( $\text{MgSO}_4$ ), and the solvent was evaporated under reduced pressure. The resultant solid was recrystallised from ethyl acetate/petroleum ether to give white crystals (146 mg, 63%) m.p. 129-130°C.

Spectral data for **2.14a**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  6.75 (t (br), 2H,  $J=5.8$  Hz, NH); 4.20 (q, 4H,  $J=6.9$  Hz,  $\text{OCH}_2$ ); 4.14 (dd, 2H,  $J=5.8, 18.0$  Hz,  $\text{NHCH}_2$ ); 3.97 (dd, 2H,  $J=5.8, 18.0$  Hz,  $\text{NHCH}_2$ ); 3.76 (s, 2H,  $\text{CH}(\text{epoxide})$ ); 1.28 (t, 6H,  $J=7.4$  Hz, Me).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  169.2 ( $\text{CO}_2$ ); 164.9 (CONH); 61.6 ( $\text{OCH}_2$ ); 55.0 ( $\text{C}(\text{epoxide})$ ); 41.0 ( $\text{NHCH}_2$ ); 14.1 (Me). HRMS Calculated for  $\text{C}_{12}\text{H}_{19}\text{N}_2\text{O}_7$  ( $\text{M}^+ + \text{H}$ ): 303.11921. Found: 303.11930. FTIR (KBr) 3332; 2924; 1743; 1681; 1556  $\text{cm}^{-1}$ . Anal. Calculated for  $\text{C}_{12}\text{H}_{18}\text{N}_2\text{O}_7$ : C 47.7, H 6.0, N 9.3. Found: C 47.4, H 6.1, N 9.2.

Preparation<sup>2.24</sup> of (2*S*,3*R*)-*N,N*-(Methyl-L-leucinyl)-2,3-epoxybutandiamide (**2.14b**)

To a stirring solution of 500 mg (3.8 mmol) of **2.16** (synthesised by a literature procedure<sup>2.25</sup>) in  $\text{CH}_2\text{Cl}_2$  (10 ml), at 0 °C, were added two equivalents (7.6 mmol) of each L-leucine ethyl ester hydrochloride (1.381 g),  $\text{Et}_3\text{N}$  (769 mg), DCC (1.568 g), and HOBT (1.027 g). The resultant solution was stirred at r.t. overnight after which the solvent was evaporated under reduced pressure. Ethyl acetate was then added to the residue to precipitate the DCU byproduct, which was removed by filtration. The filtrate was washed (3x) with saturated  $\text{NaHCO}_3$ , dried with  $\text{MgSO}_4$ , and the solvent was evaporated

under reduced pressure. The crude product was purified by radial chromatography on silica (60% dichloromethane, 40% ethyl acetate) to give a colourless oil (903 mg, 62%).

Spectral data for **2.14b**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  6.65 (d, 1H,  $J=7.3$  Hz, NH); 6.58 (d, 1H,  $J=7.8$  Hz, NH); 4.57 (m, 2H, NHCH); 3.73 (s, 3H, OMe); 3.72 (s, 3H, OMe); 3.70, 3.68 (ABq, 2H,  $J=7.3$  Hz, CH(epoxide)); 1.57 (m, 6H,  $\text{CH}_2\text{CH}(\text{Me})_2$ ); 0.92 (m, 12H, CH(Me) $_2$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  172.6 ( $\text{CO}_2$ ); 165.1, 165.0 (CONH); 55.0, 54.9 (C(epoxide)); 53.2, 52.2 (NHCH); 50.8 (OMe); 41.2, 41.1 ( $\text{CH}_2$ ); 24.7, 24.5 ( $\text{CH}(\text{Me})_2$ ); 22.6, 21.9 (CH(Me) $_2$ ). HRMS Calculated for  $\text{C}_{18}\text{H}_{31}\text{N}_2\text{O}_7$  ( $\text{M}^++\text{H}$ ): 387.21311. Found: 387.21360. FTIR (thin film) 3304; 3075; 2958; 2872; 2252; 1742; 1682; 1651; 1538  $\text{cm}^{-1}$ .

Preparation<sup>2.24</sup> of (2*S*,3*R*)-*N,N*-(Methyl-*L*-phenylalaninyl)-2,3-epoxybutandiamide (**2.14c**)

To a stirring solution of 100 mg (0.76 mmol) of **2.16** (synthesised by a literature procedure<sup>2.25</sup>) in  $\text{CH}_2\text{Cl}_2$  (5.0 ml), at 0 °C, were added two equivalents (1.5 mmol) of each *L*-phenylalanine methyl ester hydrochloride (324 mg),  $\text{Et}_3\text{N}$  (152 mg), DCC (309 mg), and HOBT (203 mg). The resultant solution was stirred at r.t. overnight after which the solvent was evaporated under reduced pressure. Ethyl acetate was then added to the residue to precipitate the DCU byproduct, which was removed by filtration. The filtrate was washed (3x) with saturated  $\text{NaHCO}_3$ , dried with  $\text{MgSO}_4$ , and the solvent was evaporated under reduced pressure. The crude product was purified by radial chromatography on silica (80% dichloromethane, 20% ethyl acetate) to give a colourless oil (172 mg, 50%).



Spectral data for **2.14c**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.27 (m, 6H, ArH); 7.12 (m, 4H, ArH); 6.65 (m(br), 2H, NH); 4.67 (m, 2H,  $\text{CHCH}_2$ ); 3.70 (s, 3H, Me); 3.66 (s, 3H, Me); 3.64, 3.62 (ABq, 2H,  $J=7.8$  Hz, CH(epoxide)); 3.08 (m, 4H,  $\text{CHCH}_2$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  171.0 ( $\text{CO}_2$ ); 164.4 (CONH); 135.8, 135.4, 129.2, 129.2, 128.7, 128.6, 127.3, 127.1 (Ar); 54.8, 54.7 (C(epoxide)); 53.4, 53.3 ( $\text{CHCH}_2$ ); 52.4, 52.3 (Me); 37.7, 37.7 ( $\text{CH}_2\text{Ph}$ ). HRMS Calculated for  $\text{C}_{24}\text{H}_{26}\text{N}_2\text{O}_7\text{K}$  ( $\text{M}^++\text{K}$ ): 493.13770. Found: 493.13770. FTIR (thin film) 3341; 3028; 2953; 1744; 1682;  $1536\text{ cm}^{-1}$ .

Preparation<sup>2,24</sup> of (2*S*,3*R*)-*N,N*-(Ethylglycinyglyciny)-2,3-epoxybutandiamide (**2.14d**)

To a stirring solution of 100 mg (0.76 mmol) of **2.16** (synthesised by a literature procedure<sup>2,25</sup>) in  $\text{CH}_2\text{Cl}_2$  (5.0 ml), at 0 °C, were added two equivalents (1.5 mmol) of each glycinyglycine ethyl ester hydrochloride (298 mg),  $\text{Et}_3\text{N}$  (152 mg), DCC (309 mg), and HOBT (203 mg). The resultant solution was stirred at r.t. overnight after which the solvent was evaporated under reduced pressure. The crude product was purified on a silica plate (eluting with 100% ethyl acetate) to give a white solid, tentatively assigned to the structure **2.14d** (35 mg, 11%) m.p. 156-159 °C.

Spectral data for **2.14d**:

$^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.73 (m(br), 2H, NH); 7.25 (m(br), 2H, NH); 4.71 (m, 4H,  $\text{NHCH}_2$ ); 4.18 (q, 4H,  $J=6.8$  Hz,  $\text{OCH}_2$ ); 4.00 (m, 4H,  $\text{NHCH}_2$ ); 3.96 (s, 2H, CH(epoxide)); 1.26 (t, 6H,  $J=6.8$  Hz, Me).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  62.7 ( $\text{OCH}_2$ ); 55.8 (C(epoxide)); 44.1, 43.3 ( $\text{NHCH}_2$ ); 14.7 (Me). FTIR (KBr) 3310; 2847; 1760;  $1634\text{ cm}^{-1}$ .

Preparation<sup>2,27</sup> of (2*S*,3*R*)-*N,N*-(Benzyl-*L*-valinyl-*L*-leucinyl)-2,3-epoxybutandiamide (**2.14e**)

To a stirring solution of 26 mg (0.20 mmol) of **2.16** (synthesised by a literature procedure<sup>2,25</sup>) in THF (1.0 ml), at 0 °C, were added two equivalents (0.40 mmol) of each **2.17** (129 mg) (also in THF (1.0 ml)) and DCC (83 mg). The resultant solution was stirred for 30 minutes, at 0 °C, and HOBT (54 mg, 0.4 mmol) was added. The reaction mixture was then stirred at r.t. overnight after which the solvent was evaporated under reduced pressure. Ethyl acetate was then added to the residue to precipitate the DCU byproduct, which was removed by filtration. The filtrate was washed (3x) with saturated NaHCO<sub>3</sub> followed by 10% hydrochloric acid (HCl), dried with MgSO<sub>4</sub>, and the solvent was evaporated under reduced pressure. The crude product was purified by radial chromatography on silica (60% petroleum ether, 40% ethyl acetate) to give a colourless oil (15 mg, 10%).

Spectral data for **2.14e**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.99 (d(br), 1H, J=9.3 Hz, NH); 7.67 (d(br), 1H, J=8.8 Hz, NHCHCH<sub>2</sub>); 7.55 (d(br), 1H, J=8.3 Hz, NHCHCH); 7.34 (m, 10H, ArH); 6.61 (d(br), 1H, J=10.3 Hz, NH); 5.07 (m, 4H, CH<sub>2</sub>Ph); 4.92 (q(br), 1H, J=8.8 Hz, NHCHCH<sub>2</sub>); 4.64 (m, 2H, NHCH); 4.46 (dd, 1H, J=5.4, 8.4 Hz, NHCHCH); 3.75, 3.69 (ABq, 2H, J=15.6 Hz, CH(epoxide)); 2.29 (m, 1H, NHCHCH); 2.10 (m, 1H, NHCHCH); 1.59-1.93 (m, 6H, CH<sub>2</sub>CH(Me)<sub>2</sub>); 0.87 (m, 24 H, Me). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 173.3, 171.9, 171.5, 171.3, 164.8, 164.4 (CO); 135.2, 135.1, 128.6, 128.6, 128.6, 128.4, 128.1 (Ar); 67.1, 66.9 (CH<sub>2</sub>Ph); 57.8, 57.6 (NHCHCH); 54.8, 54.3 (C(epoxide)); 51.8, 51.5 (NHCHCH<sub>2</sub>); 42.2, 41.8 (NHCHCH<sub>2</sub>); 32.2, 31.1 (NHCHCH); 24.8, 24.6 (CH<sub>2</sub>CH(Me)<sub>2</sub>); 23.3, 22.6, 22.2, 21.3 (CH<sub>2</sub>CH(Me)<sub>2</sub>); 19.1, 18.9, 18.6, 17.9 (CHCH(Me)<sub>2</sub>). HRMS Calculated for

$C_{40}H_{56}N_4O_9K$  ( $M^+ + K$ ): 775.36841. Found: 775.36895. FTIR (thin film) 3288; 2961; 1737; 1656; 1537  $cm^{-1}$ .

Preparation of (2*S*,3*R*)-*N,N*-(6-Carboxyhexyl)-2,3-epoxybutandiamide (**2.14f**) and (2*S*,3*R*)-*N,N*-(7-Carboxyheptyl)-2,3-epoxybutandiamide (**2.14g**)

To a stirring solution of 30 mg (0.07 mmol) of either **2.14h** or **2.14i** in methanol (MeOH) (1.4 ml), was added 0.15 ml of a 1M solution of sodium hydroxide (NaOH). The reaction mixture was stirred at r.t. overnight after which the MeOH was evaporated under reduced pressure. Water was added to the residue and the resultant solution was extracted (3x) with ethyl acetate. The aqueous layer was then acidified with 10% HCl and extracted (3x) with ethyl acetate. The combined fractions from the second extraction were dried with  $MgSO_4$  and evaporated under reduced pressure. The crude product was purified by recrystallisation from ethyl acetate to give either **2.14f** (18 mg, 67%) m.p. 50-51 °C or **2.14g** (15 mg, 52%) m.p. 95-96 °C as white crystals.

Spectral data for **2.14f**:

$^1H$  NMR ( $CD_3OD$ )  $\delta$  7.78 (t(br), 2H,  $J=5.8$  Hz, NH); 3.52 (s, 2H, CH(epoxide)); 3.01 (m, 4H,  $NHCH_2$ ); 2.10 (t, 4H,  $J=7.4$  Hz,  $CH_2CO$ ); 1.42 (m, 4H,  $CH_2CH_2CO$ ); 1.31 (m, 4H,  $NHCH_2CH_2$ ); 1.17 (m, 8H,  $NHCH_2CH_2(CH_2)_2$ ).  $^{13}C$  NMR ( $CD_3OD$ )  $\delta$  177.9 ( $CO_2$ ); 167.8 (CONH); 56.1 (C(epoxide)); 40.5 ( $NHCH_2$ ); 35.2 ( $CH_2CO$ ); 30.5 ( $NHCH_2CH_2$ ); 30.2, 27.2, ( $NHCH_2CH_2(CH_2)_2$ ); 26.3 ( $CH_2CH_2CO$ ). HRMS Calculated for  $C_{18}H_{30}N_2O_7K$  ( $M^+ + K$ ): 425.16898. Found: 425.16910. FTIR (thin film) 3474; 2934; 2360; 1655  $cm^{-1}$ .

Spectral data for **2.14g**:

$^1H$  NMR ( $CD_3OD$ )  $\delta$  7.77 (t(br), 2H,  $J=5.8$  Hz, NH); 3.51 (s, 2H, CH(epoxide)); 3.00 (m, 4H,  $NHCH_2$ ); 2.09 (t, 4H,  $J=7.3$  Hz,  $CH_2CO$ ); 1.41 (m, 4H,  $CH_2CH_2CO$ ); 1.29 (m, 4H,

NHCH<sub>2</sub>CH<sub>2</sub>); 1.15 (m, 12H, NHCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>). <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 177.9 (CO<sub>2</sub>); 167.8 (CONH); 56.1 (C(epoxide)); 40.5 (NHCH<sub>2</sub>); 35.2 (CH<sub>2</sub>CO); 30.6 (NHCH<sub>2</sub>CH<sub>2</sub>); 30.4, 30.4, 28.0 (NHCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>); 26.3 (CH<sub>2</sub>CH<sub>2</sub>CO). HRMS Calculated for C<sub>20</sub>H<sub>35</sub>N<sub>2</sub>O<sub>7</sub> (M<sup>+</sup>+H): 415.24441 Found: 415 24450. FTIR (thin film) 3455; 2932; 2360; 1638 cm<sup>-1</sup>.

Preparation<sup>2,24</sup> of (2*S*,3*R*)-*N,N*-(6-Methoxycarbonylhexyl)-2,3-epoxybutandiamide (**2.14h**) and (2*S*,3*R*)-*N,N*-(7-Methoxycarbonylheptyl)-2,3-epoxybutandiamide (**2.14i**)

To a stirring solution of 250 mg (1.9 mmol) of **2.16** (synthesised by a literature procedure<sup>2,25</sup>) in CH<sub>2</sub>Cl<sub>2</sub> (15 ml), at 0 °C, were added two equivalents (3.8 mmol) of each **2.18**<sup>2,28, 2.29</sup> (either n=6 (744 mg) or n=7 (797 mg)), Et<sub>3</sub>N (385 mg), DCC (784 mg), and HOBT (513 mg). The resultant solution was stirred at r.t. overnight after which the solvent was evaporated under reduced pressure. Ethyl acetate was then added to the residue to precipitate the DCU byproduct, which was removed by filtration. The filtrate was washed (3x) with saturated NaHCO<sub>3</sub>, dried with MgSO<sub>4</sub>, and the solvent was evaporated under reduced pressure. The crude product was recrystallised from ethyl acetate/petroleum ether to give either **2.14h** (406 mg, 52%) m.p. 45-46 °C or **2.14i** (451 mg, 54%) m.p. 47-48 °C as white crystals.

Spectral data for **2.14h**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.70 (t(br), 2H, J=5.8 Hz, NH); 3.64 (s, 6H, OMe); 3.61 (s, 2H, CH(epoxide)); 3.20 (q, 4H, J=5.8 Hz, NHCH<sub>2</sub>); 2.28 (t, 4H, J=7.4 Hz, CH<sub>2</sub>CO); 1.59 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>CO); 1.47 (m, 4H, NHCH<sub>2</sub>CH<sub>2</sub>); 1.30 (m, 8H, NHCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>).  
<sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 175.2 (CO<sub>2</sub>); 165.1 (CONH); 54.9 (C(epoxide)); 51.5 (OMe); 39.3 (NHCH<sub>2</sub>); 33.9 (CH<sub>2</sub>CO); 29.1 (NHCH<sub>2</sub>CH<sub>2</sub>); 28.6, 26.4 (NHCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>); 24.7

( $\underline{\text{CH}_2\text{CH}_2\text{CO}}$ ). HRMS Calculated for  $\text{C}_{20}\text{H}_{34}\text{N}_2\text{O}_7\text{K}$  ( $\text{M}^+\text{+K}$ ): 453 20029. Found: 453 20046. FTIR (thin film) 3302; 2935; 2859; 1679; 1555  $\text{cm}^{-1}$ . Anal. Calculated for  $\text{C}_{20}\text{H}_{34}\text{N}_2\text{O}_7$ : C 58.0, H 8.3, N 6.8. Found: C 57.9, H 8.0, N 6.8.

Spectral data for **2.14i**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  6.71 (t(br), 2H,  $J=5.9$  Hz, NH); 3.63 (s, 6H, OMe); 3.60 (s, 2H, CH(epoxide)); 3.19 (q, 4H,  $J=5.9$  Hz,  $\text{NHCH}_2$ ); 2.27 (t, 4H,  $J=7.3$  Hz,  $\text{CH}_2\text{CO}$ ); 1.58 (m, 4H,  $\text{CH}_2\text{CH}_2\text{CO}$ ); 1.45 (m, 4H,  $\text{NHCH}_2\text{CH}_2$ ); 1.27 (m, 12H,  $\text{NHCH}_2\text{CH}_2(\text{CH}_2)_3$ ).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  174.3 ( $\text{CO}_2$ ); 165.1 (CONH); 54.9 (C(epoxide)); 51.5 (OMe); 39.4 ( $\text{NHCH}_2$ ); 34.0 ( $\text{CH}_2\text{CO}$ ); 29.2 ( $\text{NHCH}_2\text{CH}_2$ ); 28.9, 28.8 26.5 ( $\text{NHCH}_2\text{CH}_2(\text{CH}_2)_3$ ); 24.8 ( $\text{CH}_2\text{CH}_2\text{CO}$ ). HRMS Calculated for  $\text{C}_{22}\text{H}_{38}\text{N}_2\text{O}_7\text{K}$  ( $\text{M}^+\text{+K}$ ): 481.23159. Found: 481.23170. FTIR (thin film) 3315; 2932; 2856; 1738; 1659; 1553  $\text{cm}^{-1}$ . Anal. Calculated for  $\text{C}_{22}\text{H}_{38}\text{N}_2\text{O}_7$ : C 59.7, H 8.7, N 6.3. Found: C 59.6, H 8.7, N 6.4.

Preparation<sup>2,24</sup> of (2*S*,3*R*)-*N,N*-Heptyl-2,3-epoxybutandiamide (**2.14j**) and (2*S*,3*R*)-*N,N*-Octyl-2,3-epoxybutandiamide (**2.14k**)

To a stirring solution of 50 mg (0.38 mmol) of **2.16** (synthesised by a literature procedure<sup>2,25</sup>) in  $\text{CH}_2\text{Cl}_2$  (15 ml), at 0 °C, were added two equivalents (0.76 mmol) of each **2.19** (either  $n=6$  (88 mg) or  $n=7$  (98 mg)), DCC (157 mg), and HOBT (103 mg). The resultant solution was stirred at r.t. overnight after which the solvent was evaporated under reduced pressure. Ethyl acetate was then added to the residue to precipitate the DCU byproduct, which was removed by filtration. The filtrate was washed (3x) with saturated  $\text{NaHCO}_3$ , dried with  $\text{MgSO}_4$ , and the solvent was evaporated under reduced pressure. The crude product was recrystallised from ethyl acetate/petroleum ether to give

either **2.14j** (96 mg, 77%) m.p. 82-83 °C or **2.14k** (80 mg, 59%) m.p. 83-84 °C, as white crystals.

Spectral data for **2.14j**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  6.65 (t(br), 2H,  $J=5.8$  Hz, NH); 3.62 (s, 2H, CH(epoxide)); 3.21 (q, 4H,  $J=5.8$  Hz,  $\text{NHCH}_2$ ); 1.46 (m, 4H,  $\text{NHCH}_2\text{CH}_2$ ); 1.26 (m, 16H,  $(\text{CH}_2)_4\text{CH}_3$ ); 0.86 (t, 6H,  $J=6.8$  Hz, Me).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  164.9 (CONH); 55.0 (C(epoxide)); 39.5 ( $\text{NHCH}_2$ ); 31.7 ( $\text{NHCH}_2\text{CH}_2$ ); 29.4, 28.9, 26.7 ( $\text{NHCH}_2\text{CH}_2(\text{CH}_2)_3$ ); 22.6 ( $\text{CH}_2\text{Me}$ ); 14.9 (Me). HRMS Calculated for  $\text{C}_{18}\text{H}_{34}\text{N}_2\text{O}_3$ : 326.25693. Found: 326.2569. FTIR (thin film) 3280; 2956; 2928; 2856; 1661; 1558  $\text{cm}^{-1}$ . Anal. Calculated for  $\text{C}_{18}\text{H}_{34}\text{N}_2\text{O}_3$ : C 66.2, H 10.5, N 8.6. Found: C 65.9, H 10.4, N 8.7.

Spectral data for **2.14k**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  6.50 (t(br), 2H,  $J=5.8$  Hz, NH); 3.62 (s, 2H, CH(epoxide)); 3.21 (q, 4H,  $J=5.8$  Hz,  $\text{NHCH}_2$ ); 1.46 (m, 4H,  $\text{NHCH}_2\text{CH}_2$ ); 1.25 (m, 20H,  $(\text{CH}_2)_5\text{Me}$ ); 0.86 (t, 6H,  $J=6.8$  Hz, Me).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  165.0 (CONH); 54.9 (C(epoxide)); 39.4 ( $\text{NHCH}_2$ ); 31.7 ( $\text{NHCH}_2\text{CH}_2$ ); 29.3, 29.2, 29.1, 26.8 ( $\text{NHCH}_2\text{CH}_2(\text{CH}_2)_4$ ); 22.6 ( $\text{CH}_2\text{Me}$ ); 14.0 (Me). HRMS Calculated for  $\text{C}_{20}\text{H}_{38}\text{N}_2\text{O}_3$ : 354.28822. Found: 354.28885. FTIR (thin film) 3278; 3098; 2957; 2926; 2426; 2122; 1660; 1557  $\text{cm}^{-1}$ . Anal. Calculated for  $\text{C}_{20}\text{H}_{38}\text{N}_2\text{O}_3$ : C 67.8, H 10.8, N 7.9. Found: C 67.8, H 10.7, N 8.0.

### Preparation<sup>2.27, 2.30, 2.50</sup> of L-Leucinyl-L-valine benzyl ester (**2.17**)

#### Step 1

To a stirring solution of 690 mg (3.0 mmol) of *N*-Boc-L-leucine in tetrahydrofuran (THF) (5.0 ml), at 0 °C, were added 3.0 mmol of each L-valine benzyl ester hydrochloride (730 mg),  $\text{Et}_3\text{N}$  (304 mg), DCC (618 mg), and HOBT (406 mg). The resultant solution

was stirred at r.t. overnight after which the solvent was evaporated under reduced pressure. Ethyl acetate was then added to the residue to precipitate the DCU byproduct, which was removed by filtration. The filtrate was washed (3x) with saturated  $\text{NaHCO}_3$ , dried with  $\text{MgSO}_4$ , and the solvent was evaporated under reduced pressure. The crude product was purified by radial chromatography on silica (80% petroleum ether, 20% ethyl acetate) to give the intermediate, *N*-Boc-L-leuciny-L-valine benzyl ester (800 mg, 63%).

## Step 2

A solution of 400 mg (0.96 mmol) of *N*-Boc-L-leuciny-L-valine benzyl ester in formic acid (1.6 ml) was stirred overnight, at r.t., after which the formic acid was evaporated under reduced pressure. Water (2.0 ml) was added to the residue and the resultant solution was alkalified (pH=10) with 25% NaOH and then extracted (3x) with ethyl acetate. The combined organic layers were dried with  $\text{MgSO}_4$  and evaporated to give **2.17** as a colourless oil (129 mg, 42%).

Spectral data for the intermediate, *N*-Boc-L-Leuciny-L-valine benzyl ester:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.35 (m, 5H, ArH); 6.54 (d, 1H,  $J=8.8$  Hz, NH); 5.20, 5.12 (ABq, 2H,  $J=12.2$  Hz,  $\text{CH}_2\text{Ph}$ ); 4.85 (d(br),  $J=7.1$  Hz, NH); 4.57 (dd, 1H,  $J=4.9, 8.8$  Hz,  $\text{NHCHCH}$ ); 4.10 (m, 1H,  $\text{NHCHCH}_2$ ); 2.19 (m, 1H,  $\text{CHCH}(\text{Me})_2$ ); 1.63 (m, 3H,  $\text{CH}_2\text{CH}(\text{Me})_2$ ); 1.43 (s, 9H,  $\text{C}(\text{Me})_3$ ); 0.89 (m, 12H, Me).

Spectral data for **2.17**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.82 (d(br), 1H,  $J=8.8$  Hz, NH); 7.35 (m, 5H, ArH); 5.20, 5.13 (ABq, 2H,  $J=12.2$  Hz,  $\text{CH}_2\text{Ph}$ ); 4.57 (dd, 1H,  $J=4.4, 7.8$  Hz,  $\text{NHCHCH}$ ); 3.44 (m, 1H,  $\text{NHCHCH}_2$ ); 2.19 (m, 1H,  $\text{CHCH}(\text{Me})_2$ ); 1.72 (m, 2H,  $\text{NHCHCH}_2$ ); 1.35 (m, 1H,  $\text{CH}_2\text{CH}(\text{Me})_2$ ); 0.94 (m, 12H, Me).

Preparation of (2*RS*,3*SR*)-3-(Heptylcarbamoyl)-2,3-epoxypropanoic acid (**2.20**)

To a stirring solution of 15 mg (0.06 mmol) of **2.22** in MeOH (1.2 ml), was added 0.06 ml of a 1M solution of NaOH. The reaction mixture was stirred at r.t. overnight after which the MeOH was evaporated under reduced pressure. Water was added to the residue and the resultant solution was extracted (3x) with ethyl acetate. The aqueous layer was then acidified with 10% HCl and extracted (3x) with ethyl acetate. The combined fractions from the second extraction were dried with MgSO<sub>4</sub> and evaporated under reduced pressure. The crude product was purified by recrystallisation from ethyl acetate/petroleum ether to give (**2.20**) (7 mg, 50%) m.p. 87-89 °C as white crystals.

Spectral data for **2.20**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.43 (t(br), 1H, J=5.8 Hz, NH); 3.75, 3.68 (ABq, 2H, J=4.9 Hz, CH(epoxide)); 3.17 (m, 2H, NHCH<sub>2</sub>); 1.48 (m, 2H, NHCH<sub>2</sub>CH<sub>2</sub>); 1.30 (m, 8H, (CH<sub>2</sub>)<sub>4</sub>Me); 0.89 (t, 3H, J=6.7 Hz, Me). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 169.7, 168.0 (CO); 55.5, 54.6 (C(epoxide)); 40.5 (NHCH<sub>2</sub>); 33.2 (NHCH<sub>2</sub>CH<sub>2</sub>); 30.6, 30.4, 28.2 (NHCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>); 24.0 (CH<sub>2</sub>Me); 14.7 (Me). HRMS Calculated for C<sub>11</sub>H<sub>19</sub>NO<sub>4</sub>: 229.13140. Found: 229.1319. FTIR (thin film) 3313; 2923; 2364; 1746 cm<sup>-1</sup>.

Preparation<sup>2,27</sup> of (2*RS*,3*SR*)-Ethyl-3-(heptylcarbamoyl)-2,3-epoxypropanoate (**2.22**)

To a stirring solution of 88 mg (0.56 mmol) of **2.21** (synthesised by a literature procedure<sup>2,30</sup>) in THF (1.0 ml), at 0 °C, was added one equivalent (88 mg, 0.56 mmol) of **2.19** (n=6), in THF (1.0 ml), followed by DCC (112 mg, 0.56 mmol). The resultant solution was stirred for 30 minutes, at 0 °C, and HOBT (72 mg, 0.4 mmol) was added. The reaction mixture was then stirred at r.t. overnight after which the solvent was



evaporated under reduced pressure. Ethyl acetate was then added to the residue to precipitate the DCU byproduct, which was removed by filtration. The filtrate was washed (3x) with saturated NaHCO<sub>3</sub>, dried with MgSO<sub>4</sub>, and the solvent was evaporated under reduced pressure. The crude product was recrystallised from ethyl acetate/petroleum ether to give (**2.22**) (56 mg, 39%) m.p. 30-31 °C as white crystals.

Spectral data for **2.22**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.44 (t(br), 1H, J=5.8 Hz, NH); 4.22 (dq, 2H, J=2.0, 4.8 Hz, CH<sub>2</sub>O); 3.70, 3.68 (ABq, 2H, J=4.9 Hz, CH(epoxide)); 3.23 (m, 2H, NHCH<sub>2</sub>); 1.48 (m, 2H, NHCH<sub>2</sub>CH<sub>2</sub>); 1.29 (m, 8H, (CH<sub>2</sub>)<sub>4</sub>Me); 1.29 (t, 3H, J=7.3 Hz, MeCH<sub>2</sub>O); 0.86 (t, 3H, J=6.9 Hz, (CH<sub>2</sub>)<sub>6</sub>Me). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 166.1, 164.5 (CO); 62.2 (CH<sub>2</sub>O); 54.7, 53.4 (C(epoxide)); 39.1 (NHCH<sub>2</sub>); 31.7 (NHCH<sub>2</sub>CH<sub>2</sub>); 29.3, 28.8, 26.7 (NHCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>); 22.5 (CH<sub>2</sub>Me); 14.0 (Me). HRMS Calculated for C<sub>13</sub>H<sub>23</sub>NO<sub>4</sub>: 257.16270. Found: 257.1624. FTIR (thin film) 3323; 2929; 2857; 2360; 2341; 1749; 1673; 1544 cm<sup>-1</sup>.

#### General Procedure<sup>2,39</sup> for the Preparation of Compounds **2.23a** and **2.23b**

To a stirring solution of 15 mg (0.08 mmol) of **2.29a** in CH<sub>2</sub>Cl<sub>2</sub> (1.5 ml), were added 0.08 mmol of the amino acid, of benzotriazolyloxy-tris-(dimethylamino)phosphonium hexafluorophosphate (BOP) (35 mg) and of Et<sub>3</sub>N (16 mg). The resultant solution was stirred for 3 hours after which the solvent was removed. The residue was dissolved in ethyl acetate, washed with water (3x), and then washed with brine (2x). The organic layer was dried (MgSO<sub>4</sub>) and evaporated under reduced pressure.

Preparation<sup>2,39</sup> of (2*R*,3*R*,1'*S*)-*N*-(1-Methoxycarbonyl-3-methylbutyl)-2,3-epoxy-5-phenylpentamide (2.23a)

The reaction was performed as described by the general procedure, with 15 mg (0.08 mmol) of L-leucine methyl ester hydrochloride. Isolation by radial chromatography on silica (70% petroleum ether, 30% ethyl acetate) gave **2.23a** as white crystals (15 mg, 59%) m.p. 43-44 °C.

Spectral data for **2.23a**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.23 (m, 5H, ArH); 6.45 (d, 1H, J=8.8 Hz, NH); 4.66 (dt, 1H, J=4.4, 9.3 Hz, NHCH); 3.63 (s, 3H, OMe); 3.53 (d, 1H, J=4.9 Hz, CHCHCO); 3.21 (dt, 1H, J=4.9, 6.4 Hz, CHCHCO); 2.82 (m, 2H, PhCH<sub>2</sub>); 1.99 (q, 2H, J=6.8 Hz, PhCH<sub>2</sub>CH<sub>2</sub>); 1.55-1.70 (m, 3H, CH<sub>2</sub>CH(Me)<sub>2</sub>); 0.95 (m, 6H, CH(Me)<sub>2</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 172.2 (CO<sub>2</sub>); 166.6 (CONH); 140.1, 128.0, 128.0, 125.6 (Ar); 57.7 (NHCH); 54.6 (OMe); 51.7 (CHCHCO); 49.4 (CHCHCO); 40.7 (NHCHCH<sub>2</sub>); 31.7 (PhCH<sub>2</sub>); 28.4 (PhCH<sub>2</sub>CH<sub>2</sub>); 24.2 (CH(Me)<sub>2</sub>); 22.3, 22.1 (CH(Me)<sub>2</sub>). HRMS Calculated for C<sub>18</sub>H<sub>25</sub>NO<sub>4</sub>: 319.17836. Found: 319.17807. FTIR (CHCl<sub>3</sub>) 3408; 2960; 1742; 1678; 1526 cm<sup>-1</sup>. [α]<sub>D</sub><sup>23</sup> +2.8 (c 5.0, CHCl<sub>3</sub>).

Preparation<sup>2,39</sup> of (2*R*,3*R*,1'*S*)-*N*-(1-Methoxycarbonyl-2-methylpropyl)-2,3-epoxy-5-phenylpentamide (2.23b)

The reaction was performed as described by the general procedure, with 13 mg (0.08 mmol) of L-valine methyl ester hydrochloride. Isolation by radial chromatography on silica (70% petroleum ether, 30% ethyl acetate) gave **2.23b** as a colourless oil (14 mg, 57%).

Spectral data for **2.23b**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.24 (m, 5H, ArH); 6.58 (d, 1H,  $J=9.3$  Hz, NH); 4.57 (dd, 1H,  $J=4.9$ , 9.3 Hz, NHCH); 3.64 (s, 3H, OMe); 3.55 (d, 1H,  $J=4.9$  Hz,  $\text{CH}_2\text{CHCH}$ ); 3.22 (m, 1H,  $\text{CH}_2\text{CH}$ ); 2.81 (m, 2H,  $\text{PhCH}_2$ ); 2.21 (m, 1H,  $\text{CH}(\text{Me})_2$ ); 1.95 (q, 2H,  $J=7.8$  Hz,  $\text{CH}_2\text{CH}$ ); 0.97 (d, 3H,  $J=6.8$  Hz,  $\text{CH}(\text{Me})_2$ ); 0.91 (d, 3H,  $J=7.3$  Hz,  $\text{CH}(\text{Me})_2$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  172.2 ( $\text{CO}_2$ ); 166.7 (CONH); 140.1, 127.9, 127.9, 125.6 (Ar); 57.6 (NHCH); 55.9 (OMe); 54.6 ( $\text{CH}_2\text{CHCH}$ ); 51.6 ( $\text{CH}_2\text{CH}$ ); 31.7 ( $\text{PhCH}_2$ ); 30.4 ( $\text{CH}(\text{Me})_2$ ); 28.5 ( $\text{CH}_2\text{CH}$ ); 18.5, 17.0 ( $\text{CH}(\text{Me})_2$ ). HRMS Calculated for  $\text{C}_{17}\text{H}_{23}\text{NO}_4$ : 305.16271. Found: 305.16278. FTIR ( $\text{CHCl}_3$ ) 3411; 2969; 1740; 1678; 1522  $\text{cm}^{-1}$ .  $[\alpha]_{\text{D}}^{23} +14.2$  (c 9.0,  $\text{CHCl}_3$ ).

#### General Procedure for the Preparation of Compounds **2.24a** and **2.24b**

To a stirring solution of 10 mg (0.04 mmol) of **2.29b** in  $\text{CH}_2\text{Cl}_2$  (1.0 ml), were added 0.04 mmol of the amino acid, of BOP (19 mg), and of  $\text{Et}_3\text{N}$  (4 mg). The resultant solution was stirred for 3 hours after which the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate, washed with water (3x), and then washed with brine (2x). The organic layer was dried ( $\text{MgSO}_4$ ) and evaporated under reduced pressure.

#### Preparation<sup>2,39</sup> of (2*R*,3*R*,1'*S*)-*N*-(1-Methoxycarbonyl-3-methylbutyl)-2,3-epoxy-4-(4-nitrophenoxy)butamide (**2.24a**)

The reaction was performed as described by the general procedure, with 8 mg (0.04 mmol) of L-leucine methyl ester hydrochloride. Isolation by radial chromatography

on silica (60% petroleum ether, 40% ethyl acetate) gave **2.24a** as white crystals (8 mg, 53%) m.p. 91-92 °C.

Spectral data for **2.24a**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.22 (d, 2H, J=9.3 Hz, ArH); 7.06 (d, 2H, J=9.3 Hz, ArH); 6.44 (d, 1H, J=9.3 Hz, NH); 4.64 (dt, 1H, J=4.4, 9.8 Hz, NHCH); 4.39 (m, 2H, OCH<sub>2</sub>); 3.72 (d, 1H, J=4.9 Hz, CH<sub>2</sub>CHCH); 3.72 (s, 3H, OMe); 3.67 (m, 1H, CH<sub>2</sub>CHCH); 1.52-1.71 (m, 3H, CH<sub>2</sub>CH(Me)<sub>2</sub>); 0.95 (d, 6H, J=6.4 Hz, (Me)<sub>2</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 172.5 (CO<sub>2</sub>); 165.6 (CONH); 162.7 (COCH<sub>2</sub>); 141.4 (NO<sub>2</sub>C); 125.4 (NO<sub>2</sub>CCH); 114.2 (CHCOCH<sub>2</sub>); 65.9 (OCH<sub>2</sub>); 55.2 (NHCH); 52.8 (OMe); 52.0 (CH<sub>2</sub>CHCH); 49.7 (CH<sub>2</sub>CHCH); 40.0 (CH<sub>2</sub>CH(Me)<sub>2</sub>); 23.4 (CH(Me)<sub>2</sub>); 22.3, 20.8 ((Me)<sub>2</sub>). HRMS Calculated for C<sub>17</sub>H<sub>22</sub>N<sub>2</sub>O<sub>7</sub>: 366.14270. Found: 366.14204. FTIR (CHCl<sub>3</sub>) 3408; 2960; 1742; 1678; 1526 cm<sup>-1</sup>. [α]<sub>D</sub><sup>23</sup> +18.0 (c 2.0, CHCl<sub>3</sub>).

Preparation<sup>2,39</sup> of (2R,3R,1'S)-N-(1-Methoxycarbonyl-2-methylpropyl)-2,3-epoxy-4-(4-nitrophenoxy)butamide (**2.24b**)

The reaction was performed as described by the general procedure, with 8 mg (0.04 mmol) of L-valine methyl ester hydrochloride. Isolation by radial chromatography on silica (50% petroleum ether, 50% ethyl acetate) gave **2.24b** as white crystals (13 mg, 81%) m.p. 86-88 °C.

Spectral data for **2.24b**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.14 (d, 2H, J=9.0 Hz, ArH); 6.97 (d, 2H, J=9.5 Hz, ArH); 6.47 (d, 1H, J=8.0 Hz, NH); 4.49 (dd, 1H, J=4.5, 9.5 Hz, NHCH); 4.29 (d, 2H, J=4.5 Hz, OCH<sub>2</sub>); 3.68 (d, 1H, J=5.5 Hz, CH<sub>2</sub>CHCH); 3.65 (s, 3H, OMe) 3.60 (m, 1H, CH<sub>2</sub>CH); 2.20 (m, 1H, CH(Me)<sub>2</sub>); 0.91 (d, 3H, J=7.1 Hz, CHMe); 0.83 (d, 3H, J=7.0 Hz, CHMe).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  171.5 ( $\text{CO}_2$ ); 165.7 ( $\text{CONH}$ ); 162.6 ( $\text{COCH}_2$ ); 141.4 ( $\text{NO}_2\text{C}$ ); 125.4 ( $\text{NO}_2\text{CCH}$ ); 114.2 ( $\text{CHCOCH}_2$ ); 65.8 ( $\text{OCH}_2$ ); 56.1 ( $\text{NHCH}$ ); 55.2 ( $\text{OMe}$ ); 52.8 ( $\text{CH}_2\text{CHCH}$ ); 51.9 ( $\text{CH}_2\text{CH}$ ); 29.8 ( $\text{CH}(\text{Me})_2$ ); 18.6, 16.9 ( $(\text{Me})_2$ ). HRMS Calculated for  $\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}_7$ : 352.12705. Found: 352.12720. FTIR ( $\text{CHCl}_3$ ) 3417; 2968; 2360; 1740; 1686, 1594, 1518  $\text{cm}^{-1}$ .  $[\alpha]_{\text{D}}^{23} +17.1$  (c 3.5,  $\text{CHCl}_3$ ).

#### General Procedure<sup>2,34</sup> for Preparation of Compounds **2.26a-d**

A stirring solution of 2.000 g (6.6 mmol) of methyl diphenylphosphonoacetate in dry THF (20 ml) was cooled to 0 °C under a nitrogen atmosphere and 3.4 ml (6.6 mmol) of a 2.0 M solution of *n*-butyllithium in pentane was added dropwise. After 10 minutes, the reaction was cooled to -78 °C and the aldehyde (5.4 mmol) was added dropwise. The resultant mixture was stirred for 1.5 hours and the reaction was then quenched by the addition of water (20 ml) and ether (40 ml). The aqueous layer was removed and the organic layer was washed with saturated brine, dried ( $\text{MgSO}_4$ ), filtered, and the solvent was evaporated under reduced pressure.

#### Preparation<sup>2,34</sup> of (Z)-Methyl 5-phenyl-2-pentenoate (**2.26a**) and (E)-Methyl 5-phenyl-2-pentenoate (**2.26c**)

The reaction was performed as described by the general procedure, with 736 mg (5.4 mmol) of the aldehyde (**2.25a**). The *E*:*Z* ratio of the crude product was found to be 1:6.4. Isolation by radial chromatography on silica (95% petroleum ether, 5% ethyl acetate) gave **2.26a** (804 mg, 78%) and **2.26c** (120 mg, 12%) as colourless oils.

Spectral data for **2.26a**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.19-7.31 (m, 5H, ArH); 6.26 (dt, 1H,  $J=7.3, 11.7$  Hz,  $\text{CH}_2\text{CH}$ ); 5.79 (dt, 1H,  $J=2.0, 11.7$  Hz,  $\text{CHCO}$ ); 3.70 (s, 3H, Me); 2.98 (dq, 2H,  $J=2.0, 7.8$  Hz,  $\text{CH}_2\text{CH}$ ); 2.77 (t, 2H,  $J=7.3$  Hz,  $\text{PhCH}_2$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  166.3 ( $\text{CO}_2$ ); 149.0 ( $\text{CH}_2\text{CH}$ ); 140.8, 128.2, 128.0, 125.7 (Ar); 119.6 ( $\text{CHCO}$ ); 50.7 (Me); 34.7, 30.2 ( $\text{CH}_2$ ). HRMS Calculated for  $\text{C}_{12}\text{H}_{14}\text{O}_2$ : 190.09938. Found: 190.09937. FTIR (thin film) 3427; 3086; 3063; 3028; 2999; 2949; 2926; 2858; 1720; 1645; 1603  $\text{cm}^{-1}$ .

Spectral data for **2.26c**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.17-7.32 (m, 5H, ArH); 7.01 (dt, 1H,  $J=6.8, 15.3$  Hz,  $\text{CH}_2\text{CH}$ ); 5.85 (dt, 1H,  $J=1.5, 15.6$  Hz,  $\text{CHCO}$ ); 3.72 (s, 3H, Me); 2.78 (t, 2H,  $J=8.3$  Hz,  $\text{PhCH}_2$ ); 2.53 (dq, 2H,  $J=1.9, 6.8$  Hz,  $\text{CH}_2\text{CH}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  166.5 ( $\text{CO}_2$ ); 148.0 ( $\text{CH}_2\text{CH}$ ); 140.4, 128.2, 128.0, 125.9 (Ar); 121.2 ( $\text{CHCO}$ ); 50.0 (Me); 34.0, 33.5 ( $\text{CH}_2$ ). HRMS Calculated for  $\text{C}_{12}\text{H}_{14}\text{O}_2$ : 190.09938. Found: 190.09980. FTIR (thin film) 3063; 3028; 2949; 2858; 1724; 1659; 1603  $\text{cm}^{-1}$ .

Preparation<sup>2,34</sup> of (Z)-Methyl 4-(4-nitrophenoxy)-2-butenolate (**2.26b**) and (E)-Methyl 4-(4-nitrophenoxy)-2-butenolate (**2.26d**)

The reaction was performed as described by the general procedure, with 990 mg (5.4 mmol) of the aldehyde (**2.25b**) (synthesised by a literature procedure<sup>2,35</sup>). The *E*:*Z* ratio of the crude product was found to be 1:3.4. Isolation by radial chromatography on silica (80% petroleum ether, 20% ethyl acetate) gave **2.26b** (1.082 g, 84%) and **2.26d** (193 mg, 15%) as white crystals m.p. 74-75 and 120-121  $^{\circ}\text{C}$ , respectively.

Spectral data for **2.26b**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.21 (d, 2H,  $J=9.3$  Hz, ArH); 6.98 (d, 2H,  $J=9.2$  Hz, ArH); 6.47 (dt, 1H,  $J=4.8, 11.7$  Hz,  $\text{CH}_2\text{CH}$ ); 5.99 (dt, 1H,  $J=2.4, 11.7$  Hz,  $\text{CHCO}_2$ ); 5.25 (dd, 2H,  $J=2.4, 4.8$  Hz,  $\text{CH}_2$ ); 3.78 (s, 3H, Me).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  165.9 ( $\text{CO}_2$ ); 162.8 ( $\text{COCH}_2$ ); 144.7 ( $\text{CH}_2\text{CH}$ ); 140.6 ( $\text{NO}_2\text{C}$ ); 125.6 ( $\text{NO}_2\text{CCH}$ ); 120.3 ( $\text{CHCO}_2$ ); 114.3 ( $\text{CHCOCH}_2$ ); 66.5 ( $\text{CH}_2$ ); 51.3 (Me). HRMS Calculated for  $\text{C}_{11}\text{H}_{11}\text{NO}_5$ : 237.06372. Found: 237.06397. FTIR (thin film) 3453; 3115; 1717; 1663; 1590  $\text{cm}^{-1}$ . Anal. Calculated for  $\text{C}_{11}\text{H}_{11}\text{NO}_5$ : C 55.9, H 4.7, N 5.9. Found: C 55.6, H 4.8, N 5.8.

Spectral data for **2.26d**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.22 (d, 2H,  $J=9.3$  Hz, ArH); 7.07 (dt, 1H,  $J=3.9, 16.1$  Hz,  $\text{CH}_2\text{CH}$ ); 6.98 (d, 2H,  $J=9.3$  Hz, ArH); 6.19 (dt, 1H,  $J=1.9, 16.1$  Hz,  $\text{CHCO}_2$ ); 4.80 (dd, 2H,  $J=2.0, 3.9$  Hz  $\text{CH}_2$ ); 3.77 (s, 3H, Me).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  165.7 ( $\text{CO}_2$ ); 162.5 ( $\text{COCH}_2$ ); 141.6 ( $\text{CH}_2\text{CH}$ ); 140.6 ( $\text{NO}_2\text{C}$ ); 125.6 ( $\text{NO}_2\text{CCH}$ ); 122.0 ( $\text{CHCO}_2$ ); 114.3 ( $\text{CHCOCH}_2$ ); 66.6 ( $\text{CH}_2$ ); 51.5 (Me). HRMS Calculated for  $\text{C}_{11}\text{H}_{11}\text{NO}_5$ : 237.06372. Found: 237.06339. FTIR (thin film) 3084; 2912; 1713; 1651; 1609; 1593  $\text{cm}^{-1}$ .

#### Preparation<sup>2,36</sup> of (Z)-5-Phenyl-2-penten-1-ol (**2.27a**)

A stirring solution of 600 mg (3.1 mmol) of **2.26a** in dry  $\text{CH}_2\text{Cl}_2$  (24 ml) was cooled to  $-78$  °C under a nitrogen atmosphere and 7.7 ml (7.7 mmol) of a 1 M solution of diisobutylaluminium hydride (DIBAL) in  $\text{CH}_2\text{Cl}_2$  was added dropwise. The resultant solution was stirred for 3 hours at  $-78$  °C after which water (1.0 ml) was added over 5 minutes, followed by 10% potassium sodium tartrate (10 ml). The mixture was warmed to r.t. and the aqueous layer was removed. The organic layer was washed (3x) with 10% potassium sodium tartrate and the combined aqueous fractions were washed with

$\text{CH}_2\text{Cl}_2$  (3x). The combined organic fractions were dried ( $\text{MgSO}_4$ ) and the solvent was evaporated under reduced pressure. The crude product was purified by radial chromatography on silica (60% petroleum ether, 40% ethyl acetate) to give a colourless oil (470 mg, 93%).

Spectral data for **2.27a**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.16-7.36 (m, 5H, ArH); 5.58 (m, 2H, CH(olefins)); 4.00 (d, 2H,  $\text{J}=5.4$  Hz,  $\text{CH}_2\text{OH}$ ); 2.69 (t, 2H,  $\text{J}=7.8$  Hz,  $\text{PhCH}_2$ ); 2.41 (q, 2H,  $\text{J}=7.3$  Hz,  $\text{PhCH}_2\text{CH}_2$ ); 1.56 (s(br), 1H, OH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  141.5, 128.6, 128.3, 126.0 (Ar); 131.5, 129.3 (CH(olefins)); 58.3 ( $\text{CH}_2\text{OH}$ ); 35.6, 29.3 ( $\text{Ph}(\text{CH}_2)_2$ ). HRMS Calculated for  $\text{C}_{11}\text{H}_{14}\text{O}$ : 162.10447. Found: 162.10451. FTIR (thin film) 3309; 3024; 2928;  $2858\text{ cm}^{-1}$ .

#### Preparation<sup>2,36</sup> of (Z)-4-(4-Nitrophenoxy)-2-buten-1-ol (**2.27b**)

A stirring solution of 500 mg (2.1 mmol) of **2.26b** in dry  $\text{CH}_2\text{Cl}_2$  (16 ml) was cooled to  $-78^\circ\text{C}$  under a nitrogen atmosphere and 5.2 ml (5.2 mmol) of a 1 M solution of DIBAL in  $\text{CH}_2\text{Cl}_2$  was added dropwise. The resultant solution was stirred for 3 hours at  $-78^\circ\text{C}$  after which water (1.0 ml) was added over 5 minutes, followed by 10% potassium sodium tartrate (10 ml). The mixture was warmed to r.t. and the aqueous layer was removed. The organic layer was washed (3x) with 10% potassium sodium tartrate and the combined aqueous fractions were washed with  $\text{CH}_2\text{Cl}_2$  (3x). The combined organic fractions were dried ( $\text{MgSO}_4$ ) and the solvent was evaporated under reduced pressure. The crude product was purified by radial chromatography on silica (50% petroleum ether, 50% ethyl acetate) to give white crystals (257 mg, 59%) m.p.  $77-78^\circ\text{C}$ .



Spectral data for **2.27b**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.21 (d, 2H,  $J=9.3$  Hz, ArH); 6.97 (d, 2H,  $J=9.2$  Hz, ArH); 5.79-5.93 (m, 2H, CH(olefins)); 4.74 (d, 2H,  $J=5.9$  Hz,  $\text{C}_6\text{H}_4\text{OCH}_2$ ); 4.32 (t, 2H,  $J=4.9$  Hz,  $\text{CH}_2\text{OH}$ ); 1.51 (t(br), 1H,  $J=4.4$  Hz, OH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  162.9 ( $\text{COCH}_2$ ); 141.1 ( $\text{NO}_2\text{C}$ ); 132.5 (CH(olefin)); 125.4 ( $\text{NO}_2\text{CCH}$  and CH(olefin)); 114.1 ( $\text{CHCOCH}_2$ ); 64.1 ( $\text{C}_6\text{H}_4\text{OCH}_2$ ); 58.4 ( $\text{CH}_2\text{OH}$ ). HRMS Calculated for  $\text{C}_{10}\text{H}_{11}\text{NO}_4$ : 209.06881. Found: 209.06881. FTIR (thin film) 3439; 3292; 3115; 2872; 1591; 1501  $\text{cm}^{-1}$ .

Preparation<sup>2,37</sup> of (2*S*,3*R*)-2,3-Epoxy-5-phenyl-1-pentanol (**2.28a**)

A stirring solution of 701 mg (2.5 mmol) of titanium tetrakisopropoxide ( $\text{Ti}(\text{OiPr})_4$ ) and 4 Å molecular sieves (250 mg) in dry  $\text{CH}_2\text{Cl}_2$  (25 ml) was cooled to between -20 and -30°C (dry ice/carbon tetrachloride ( $\text{CCl}_4$ )) under a nitrogen atmosphere. A solution of 691 mg (3.0 mmol) of L-(+)-diisopropyl tartrate (DIPT) in dry  $\text{CH}_2\text{Cl}_2$  (1.0 ml) was added, dropwise, followed by 395 mg (2.5 mmol) of **2.27a** also in dry  $\text{CH}_2\text{Cl}_2$  (4.0 ml). The resultant solution was stirred for 30 minutes and 1.0 ml (4.8 mmol) of a 4.8 M solution of *tert*-butyl hydroperoxide (TBHP) in  $\text{CH}_2\text{Cl}_2$  was added. The resultant solution was stored in a -20 °C freezer for 20 hours. The reaction was quenched with 10% tartaric acid (25 ml), the organic layer was separated, and the aqueous layer was washed with  $\text{CH}_2\text{Cl}_2$  (3x). The combined organic fractions were dried ( $\text{MgSO}_4$ ) and evaporated under reduced pressure. The excess peroxide was removed by azeotropic distillation with  $\text{CCl}_4$  and the crude mixture was purified by radial chromatography on silica (60% petroleum ether, 40% ethyl acetate) to give a colourless oil (303 mg, 68%).

Spectral data for **2.28a**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.27 (m, 5H, ArH); 3.57 (m, 2H,  $J=5.4$  Hz,  $\text{CH}_2\text{OH}$ ); 3.11 (m, 2H, CH(epoxide)); 2.88 (m, 1H,  $\text{PhCH}_2$ ); 2.73 (m, 1H,  $\text{PhCH}_2$ ); 2.01 (m, 1H,  $\text{PhCH}_2\text{CH}_2$ ); 1.81 (m, 1H,  $\text{PhCH}_2\text{CH}_2$ ); 1.34 (m(br), 1H, OH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  140.4, 128.0, 127.9, 125.7 (Ar); 60.0 ( $\text{CH}_2\text{OH}$ ); 56.6, 56.1 (C(epoxide)); 32.2, 29.2 ( $\text{Ph}(\text{CH}_2)_2$ ). HRMS Calculated for  $\text{C}_{11}\text{H}_{12}\text{O}$  ( $\text{M}^+ - \text{H}_2\text{O}$ ): 160.08882. Found: 160.08877. FTIR (thin film) 3422(br); 3026; 2928; 2860; 1603  $\text{cm}^{-1}$ .  $[\alpha]_{\text{D}}^{23}$  -1.9 (c 10.9,  $\text{CHCl}_3$ ).

Preparation<sup>2,37</sup> of (2*S*,3*R*)-2,3-Epoxy-4-(4-nitrophenoxy)-1-butanol (**2.28b**)

A stirring solution of 266 mg (0.94 mmol) of  $\text{Ti}(\text{OiPr})_4$  and 4Å molecular sieves (94 mg) in dry  $\text{CH}_2\text{Cl}_2$  (10 ml) was cooled to between -20 and -30 °C (dry ice/  $\text{CCl}_4$ ) under a nitrogen atmosphere. A solution of 263 mg (1.1 mmol) of L-(+)-DIPT in dry  $\text{CH}_2\text{Cl}_2$  (0.5 ml) was added, dropwise, followed by 195 mg (0.94 mmol) of **2.27b** also in dry  $\text{CH}_2\text{Cl}_2$  (1.5 ml). The resultant solution was stirred for 30 minutes and 0.38 ml (1.9 mmol) of a 4.8 M solution of TBHP in  $\text{CH}_2\text{Cl}_2$  was added. The resultant solution was stored in a -20 °C freezer for 20 hours. The reaction was quenched with 10% tartaric acid (10 ml), the organic layer was separated, and the aqueous layer was washed with  $\text{CH}_2\text{Cl}_2$  (3x). The combined organic fractions were dried ( $\text{MgSO}_4$ ) and evaporated under reduced pressure. The excess peroxide was removed by azeotropic distillation with  $\text{CCl}_4$  and the crude mixture was purified by radial chromatography on silica (25% petroleum ether, 75% ethyl acetate) to give white crystals (150 mg, 71%) m.p. 114-116 °C.

Spectral data for **2.28b**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.21 (d, 2H,  $J=9.3$  Hz, ArH); 7.01 (d, 2H,  $J=9.3$  Hz, ArH); 4.37 (dd, 1H,  $J=3.4, 11.2$  Hz,  $\text{C}_6\text{H}_4\text{OCH}_2$ ); 4.28 (dd, 1H,  $J=6.9, 11.3$  Hz,  $\text{C}_6\text{H}_4\text{OCH}_2$ ); 3.98 (m, 1H,

$\text{CH}_2\text{OH}$ ); 3.87 (m, 1H,  $\text{CH}_2\text{OH}$ ); 3.47 (m, 1H,  $\text{C}_6\text{H}_4\text{OCH}_2\text{CH}$ ); 3.34 (q, 1H,  $J=4.4$  Hz,  $\text{CHCH}_2\text{OH}$ ); 1.77 (t, 1H,  $J=6.3$  Hz, OH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  167.7 ( $\text{COCH}_2$ ); 141.4 ( $\text{NO}_2\text{C}$ ); 125.4 ( $\text{NO}_2\text{CCH}$ ); 114.1 ( $\text{CHCOCH}_2$ ); 66.5 ( $\text{C}_6\text{H}_4\text{OCH}_2$ ); 59.8 ( $\text{CH}_2\text{OH}$ ); 55.3, 53.9 (C(epoxide)). HRMS Calculated for  $\text{C}_{10}\text{H}_{11}\text{NO}_5$ : 225.06372. Found: 225.06365. FTIR (thin film) 3454 (br); 3285 (br); 2930; 1593; 1510  $\text{cm}^{-1}$ .  $[\alpha]_{\text{D}}^{23}$  -13.5 (c 4.0,  $\text{CHCl}_3$ ).

Preparation<sup>2,38</sup> of (2*R*,3*R*)-2,3-Epoxy-5-phenylpentanoic acid (**2.29a**)

To a biphasic solution of  $\text{CCl}_4$  (1.0 ml), acetonitrile ( $\text{CH}_3\text{CN}$ ) (1.0 ml), and water (1.5 ml), were added **2.28a** (100 mg, 0.56 mmol), ruthenium trichloride hydrate ( $\text{RuCl}_3$ ) (2.6 mg, 2.2 mol%), and periodioic acid ( $\text{H}_5\text{IO}_6$ ) (319 mg, 1.4 mmol). The resultant solution was stirred vigorously for 3 hours at r.t. after which  $\text{CH}_2\text{Cl}_2$  was added and the organic layer was separated. The aqueous layer was washed with  $\text{CH}_2\text{Cl}_2$  (3x) and the combined organic fractions were dried ( $\text{MgSO}_4$ ) and evaporated under reduced pressure. The residue was taken up in ether, filtered through a celite pad, and concentrated to give white crystals (93 mg, 85%) m.p. 84-85 °C.

Spectral data for **2.29a**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.23 (m, 5H, ArH); 3.56 (d, 1H,  $J=4.4$  Hz,  $\text{CHCO}_2$ ); 3.26 (m, 1H,  $\text{CH}_2\text{CH}$ ); 2.80 (m, 2H,  $\text{PhCH}_2$ ); 2.00 (m, 2H,  $\text{PhCH}_2\text{CH}_2$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  173.3 ( $\text{CO}_2$ ); 139.8, 128.0, 127.9, 125.8 (Ar); 57.0, 52.0 (C(epoxide)); 31.8, 28.5 ( $\text{PhCH}_2$ ). HRMS Calculated for  $\text{C}_{11}\text{H}_{12}\text{O}_3$ : 192.07864. Found: 192.07889. FTIR ( $\text{CHCl}_3$ ) 2932; 2560; 1948; 1725; 1604  $\text{cm}^{-1}$ .  $[\alpha]_{\text{D}}^{23}$  -5.9 (c 16.0,  $\text{CHCl}_3$ ).

Preparation<sup>2,38</sup> of (2*R*,3*R*)-2,3-Epoxy-4-(4-nitrophenoxy)butanoic acid (**2.29b**)

To a biphasic solution of CCl<sub>4</sub> (0.15 ml), acetonitrile (CH<sub>3</sub>CN) (0.15 ml), and water (0.21 ml), were added **2.28b** (15 mg, 0.07 mmol), RuCl<sub>3</sub> (0.4 mg, 2.2 mol%), and H<sub>5</sub>IO<sub>6</sub> (41 mg, 0.18 mmol). The resultant solution was stirred vigorously for 3 hours at r.t. after which CH<sub>2</sub>Cl<sub>2</sub> was added and the organic layer was separated. The aqueous layer was washed with CH<sub>2</sub>Cl<sub>2</sub> (3x) and the combined organic fractions were dried (MgSO<sub>4</sub>) and evaporated under reduced pressure. The residue was taken up in ether, filtered through a celite pad, and concentrated to give white crystals (12 mg, 72%) m.p. 108-109 °C.

Spectral data for **2.29b**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.21 (d, 2H, J=6.9 Hz, ArH); 7.01 (d, 2H, J=6.8 Hz, ArH); 4.38 (m, 2H, OCH<sub>2</sub>); 3.73 (d, 1H, J=4.4 Hz, CHCO<sub>2</sub>); 3.68 (q, 1H, J=5.3 Hz, CH<sub>2</sub>CH). <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 171.9 (CO<sub>2</sub>); 166.0 (COCH<sub>2</sub>); 144.2 (NO<sub>2</sub>C); 127.8 (NO<sub>2</sub>CCH); 117.0 (CHCOCH<sub>2</sub>); 68.6 (OCH<sub>2</sub>); 56.4, 53.3 (C(epoxide)). HRMS Calculated for C<sub>10</sub>H<sub>9</sub>NO<sub>6</sub>: 239.04299. Found: 239.04320. FTIR (KBr) 3416; 2924; 2367; 1718; 1639; 1612; 1593; 1560; 1522 cm<sup>-1</sup>. [α]<sub>D</sub><sup>23</sup> +15.2 (c 13.0, THF).

General Procedure<sup>2,40</sup> for the Preparation of Compounds **2.30a** and **2.30b**

To a stirring solution of 0.10 mmol of the epoxy alcohol (either **2.28a** or **2.28b**) in CH<sub>2</sub>Cl<sub>2</sub> (0.5 ml), were added dimethylaminopyridine (12 mg, 0.10 mmol), Et<sub>3</sub>N (12 mg, 0.12 mmol), and acetylchloride (9 mg, 0.12 mmol). The resultant solution was stirred for 2 hours, filtered through a silica plug, and the filtrate was evaporated under reduced pressure.

Preparation<sup>2.40</sup> of (2*S*, 3*R*)-(2,3-Epoxy-5-phenyl)pentyl acetate (**2.30a**)

The reaction was performed as described by the general procedure, with 18 mg (0.10 mmol) of **2.28a**, to give **2.30a** as a colourless oil (20 mg, 91%).

Spectral data for **2.30a**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.25 (m, 5H, ArH); 4.15 (dd, 1H, J=4.4, 12.3 Hz, CH<sub>2</sub>O); 3.97 (dd, 1H, J=7.3, 12.2 Hz, CH<sub>2</sub>O); 3.15 (m, 1H, CH(epoxide)); 3.07 (m, 1H, CH(epoxide)); 2.82 (m, 2H, PhCH<sub>2</sub>CH<sub>2</sub>); 2.09 (s, 3H, Me); 187 (m, 2H, PhCH<sub>2</sub>).

Preparation<sup>2.40</sup> of (2*S*,3*R*)-[2,3-Epoxy-4-(4-nitrophenoxy)]butyl acetate (**2.30b**)

The reaction was performed as described by the general procedure, with 23 mg (0.10 mmol) of **2.28b**, to give **2.30b** as a colourless oil (22 mg, 88%).

Spectral data for **2.30b**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.23 (d, 2H, J=9.2 Hz, ArH); 7.01 (d, 2H, J=9.3 Hz, ArH); 4.37 (m, 2H, OCH<sub>2</sub>); 4.18 (m, 2H, OCH<sub>2</sub>); 3.47 (m, 1H, CH(epoxide)); 3.39 (m, 1H, CH(epoxide)); 2.13 (s, 3H, Me).

Shift Analysis<sup>2.40</sup> on Compounds **2.30a** and **2.30b**

To a solution of 10 mg of the acetate (either **2.30a** or **2.30b**) in 0.5 ml of benzene (d<sub>6</sub>) (in an NMR tube) was added the shift reagent, europium (111) tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorate], sequentially (10 µl portions of a solution of 30 mg of shift reagent in 0.5 ml of benzene (d<sub>6</sub>)) and the acetate Me signal was monitored by <sup>1</sup>H NMR.<sup>2.40</sup> After the addition of 250 µl of shift reagent, a second resonance for the acetate Me was not observed for either **2.30a** or **2.30b**.

Preparation of (2*R*,3*R*,1'*S*)-*N*-(1-Methoxycarbonyl-2-methylpropyl)-2,3-epoxydodecylamide (2.31)

Step 1

To a stirring solution of 30 mg (0.15 mmol) **2.32** (synthesised by a literature procedure<sup>2.18</sup>) in benzene (1.0 ml), were added dimethylformamide (1 drop) and 5 equivalents of oxalyl chloride (95 mg, 0.75 mmol). The resultant solution was stirred for 1 hour at r.t., after which the solvent was evaporated under reduced pressure to give the acid chloride (**2.33**) as a yellow oil.

Step 2

To a stirring solution of 0.15 mmol of the acid chloride (**2.33**) in CH<sub>2</sub>Cl<sub>2</sub> (0.75 ml), was added a solution of L-valine methyl ester hydrochloride (24 mg, 0.15 mmol) and Et<sub>3</sub>N (15 mg, 0.15 mmol) also in CH<sub>2</sub>Cl<sub>2</sub> (0.75 ml). The resultant solution was stirred at r.t. overnight and the crude mixture was then washed (3x) with saturated NaHCO<sub>3</sub> followed by 10% hydrochloric acid (HCl), dried with MgSO<sub>4</sub>, and the solvent was evaporated under reduced pressure. The crude product was purified by radial chromatography on silica (80% petroleum ether, 20% ethyl acetate) to give **2.31** as a colourless oil (7 mg, 14%).

Spectral data for **2.33**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.94 (d, 1H, J=4.4 Hz, CHCO); 3.34 (m, 1H, CHCH<sub>2</sub>); 1.53 (m, 4H, CH(CH<sub>2</sub>)<sub>2</sub>); 1.26 (m, 12H, (CH<sub>2</sub>)<sub>6</sub>Me); 0.87 (t, 3H, J=6.9 Hz, Me). FTIR (thin film) 2925; 2855; 2360; 1805 cm<sup>-1</sup>.

Spectral data for **2.31**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.57 (d, 1H, J=9.3 Hz, NH); 4.57 (m, 1H, CHNH); 3.73 (s, 3H, MeO); 3.54 (d, 1H, J=4.9 Hz, NHCOCH); 3.20 (m, 1H, CHCH<sub>2</sub>); 2.22 (m, 1H, CH(Me)<sub>2</sub>);

1.61 (m, 2H, CHCH<sub>2</sub>); 1.48 (m, 2H, CHCH<sub>2</sub>CH<sub>2</sub>); 1.26 (m, 12H, (CH<sub>2</sub>)<sub>6</sub>Me); 0.92 (m, 9H, Me). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 171.7 (CO<sub>2</sub>); 167.5 (CONH); 59.0 (CHNH); 56.4, 55.1 (C(epoxide)); 52.1 (MeO); 31.8, 31.0, 29.5, 29.4, 29.3, 27.2, 26.1 (CH(Me)<sub>2</sub> and CH(CH<sub>2</sub>)<sub>6</sub>); 22.6 (CH<sub>2</sub>Me); 19.0, 17.5 (CH(Me)<sub>2</sub>); 14.1 (CH<sub>2</sub>Me). HRMS Calculated for C<sub>18</sub>H<sub>33</sub>NO<sub>4</sub>: 327.24094. Found: 327.2409 FTIR (thin film) 3424; 2925; 2854; 2348; 1744; 1678 cm<sup>-1</sup>. [α]<sub>D</sub><sup>23</sup> +1.0 (c 3.5, CHCl<sub>3</sub>).

Preparation<sup>2.41</sup> of (2*S*,3*R*)-*N*-CBz-*L*-Valine (2,3-epoxy-5-phenylpentyl) ester (**2.34a**)

To a stirring solution of 15 mg (0.08 mmol) of **2.28a** in THF (1.5 ml), were added *N*-CBz-*L*-valine (46 mg, 0.19 mmol) and triphenylphosphine (46 mg, 0.18 mmol). After stirring for 5 minutes, diethyl azodicarboxylate (DEAD) (31 mg, 0.18 mmol) was added dropwise. The resultant solution was stirred for 3 hours after which CH<sub>2</sub>Cl<sub>2</sub> was added and the crude mixture was washed (3x) with saturated NaHCO<sub>3</sub>. The organic layer was dried (MgSO<sub>4</sub>) and evaporated under reduced pressure. Isolation by radial chromatography on silica (80% petroleum ether, 20% ethyl acetate) gave **2.34a** as a colourless oil (16 mg, 46%).

Spectral data for **2.34a**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.19-7.35 (m, 10H, ArH); 5.30 (d(br), 1H, J=8.8 Hz, NH); 5.11 (s, 2H, CO<sub>2</sub>CH<sub>2</sub>Ph); 4.36 (dd, 1H, J=4.9, 9.3 Hz, CHNH); 4.19 (dd, 1H, J=4.4, 12.2 Hz, CHCH<sub>2</sub>O); 4.05 (dd, 1H, J=6.8, 12.2 Hz, CHCH<sub>2</sub>O); 3.14 (m, 1H, CHCH<sub>2</sub>O); 3.06 (m, 1H, (CH<sub>2</sub>)<sub>2</sub>CH); 2.72-2.89 (m, 2H, PhCH<sub>2</sub>CH<sub>2</sub>); 2.18 (m, 1H, CH(Me)<sub>2</sub>); 1.87 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH); 0.94 (d, 3H, J=6.9 Hz, Me); 0.95 (d, 3H, J=6.9 Hz, Me). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 171.3 (COCH); 155.6 (NHCO); 140.1, 135.7, 128.0, 128.0, 127.9, 127.7, 127.6, 125.7 (Ar); 66.5 (OCH<sub>2</sub>Ph); 62.8 (CHCH<sub>2</sub>O); 58.5 (CHNH); 55.3 (CH<sub>2</sub>CH<sub>2</sub>CH); 53.1

( $\underline{\text{CHCH}_2\text{CO}}$ ); 32.1 ( $\text{Ph}\underline{\text{CH}_2\text{CH}_2}$ ); 30.7 ( $\underline{\text{CH}}(\text{Me})_2$ ); 29.7 ( $\text{PhCH}_2\underline{\text{CH}_2}$ ); 18.4, 16.9 (Me).

HRMS Calculated for  $\text{C}_{24}\text{H}_{29}\text{NO}_5$ : 411.20457. Found: 411.20493 FTIR ( $\text{CHCl}_3$ ) 3436;

2968; 1722; 1511  $\text{cm}^{-1}$ .  $[\alpha]_D^{23}$  -6.1 (c 16.0,  $\text{CHCl}_3$ ).

#### General Procedure<sup>2,41</sup> for Preparation of Compounds **2.34b** and **2.35**

To a stirring solution of 10 mg (0.04 mmol) of **2.28b** in THF (1.0 ml), were added 0.09 mmol of the amino acid and of triphenylphosphine (24 mg). After stirring for 5 minutes, DEAD (16 mg, 0.09 mmol) was added dropwise. The resultant solution was stirred for 3 hours after which  $\text{CH}_2\text{Cl}_2$  was added and the crude mixture was washed (3x) with saturated  $\text{NaHCO}_3$ . The organic layer was dried ( $\text{MgSO}_4$ ) and evaporated under reduced pressure.

#### Preparation<sup>2,41</sup> of (2*S*,3*R*)-*N*-CBz-L-Valine [2,3-epoxy-4-(4-nitrophenoxy)butyl] ester (**2.34b**)

The reaction was performed as described by the general procedure, with 25 mg (0.09 mmol) of *N*-CBz-L-valine. Isolation by radial chromatography on silica (30% petroleum ether, 70% ethyl acetate) gave **2.34b** as white crystals (16 mg, 75%) m.p. 57-58 °C.

Spectral data for **2.34b**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.21 (d, 2H,  $\text{J}=9.3$  Hz,  $\text{NO}_2\text{CCH}$ ); 7.35 (s, 5H,  $\text{CH}_2\text{Ph}$ ); 7.00 (d, 2H,  $\text{J}=9.3$  Hz,  $\text{NO}_2\text{CCHCH}$ ); 5.26 (d(br), 1H,  $\text{J}=9.3$  Hz, NH); 5.11 (s, 2H,  $\text{CH}_2\text{Ph}$ ); 4.14-4.44 (m, 5H,  $\text{OCH}_2$  and  $\text{CHNH}$ ); 3.45 (m, 1H, CH(epoxide)); 3.36 (m, 1H, CH(epoxide)); 2.19 (m, 1H,  $\underline{\text{CH}}(\text{Me})_2$ ); 0.95 (d, 3H,  $\text{J}=6.8$  Hz, Me); 0.96 (d, 3H,  $\text{J}=6.9$  Hz, Me).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 171.3 (COCH); 162.5 (COCH<sub>2</sub>); 155.6 (NHCO); 141.5 ( $\text{NO}_2\text{C}$ );



135.6, 128.0, 127.7, 127.5 ( $\text{CH}_2\text{Ph}$ ); 125.4 ( $\text{NO}_2\text{CCH}$ ); 114.1 ( $\text{NO}_2\text{CCHCH}$ ); 66.6 ( $\text{CH}_2\text{Ph}$ ); 66.2 ( $\text{C}_6\text{H}_4\text{OCH}_2$ ); 61.2 ( $\text{CHCH}_2\text{OCO}$ ); 58.6 ( $\text{CHNH}$ ); 53.3 ( $\text{CHCH}_2\text{OCO}$ ); 52.3 ( $\text{C}_6\text{H}_4\text{OCH}_2\text{CH}$ ); 30.6 ( $\text{CH}(\text{Me})_2$ ); 18.4, 17.0 (Me). HRMS Calculated for  $\text{C}_{23}\text{H}_{26}\text{N}_2\text{O}_8$ : 458.16892. Found: 458.16826. FTIR ( $\text{CHCl}_3$ ) 3436; 2968; 1722; 1511  $\text{cm}^{-1}$ .  $[\alpha]_{\text{D}}^{23} +2.5$  (c 8.0,  $\text{CHCl}_3$ ).

Preparation of (2*S*,3*R*)-*N*-CBz-L-Valinyl-L-valine [2,3-epoxy-4-(4-nitrophenoxy)butyl] ester (**2.35**)

The reaction was performed as described by the general procedure, with 34 mg (0.09 mmol) of *N*-CBz-L-Valinyl-L-valine. Isolation by radial chromatography on silica (30% petroleum ether, 70% ethyl acetate) gave **2.35** as white crystals (11 mg, 45%) m.p. 92-93 °C.

Spectral data for **2.35**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.21 (d, 2H,  $J=8.8$  Hz,  $\text{NO}_2\text{CCH}$ ); 7.33 (s, 5H,  $\text{CH}_2\text{Ph}$ ); 7.00 (d, 2H,  $J=9.3$  Hz,  $\text{NO}_2\text{CCHCH}$ ); 6.44 (m(br), 1H, NH); 5.37 (d(br), 1H,  $J=8.3$  Hz, NH); 5.11 (s, 2H,  $\text{CH}_2\text{Ph}$ ); 4.55 (dd, 1H,  $J=4.9, 8.3$  Hz,  $\text{CHNH}$ ); 4.14-4.45 (m, 4H,  $\text{OCH}_2$ ); 4.04 (t(br), 1H,  $J=7.3$  Hz,  $\text{CHNH}$ ); 3.45 (m, 1H,  $\text{CHCH}_2\text{OCO}$ ); 3.36 (m, 1H,  $\text{C}_6\text{H}_4\text{OCH}_2\text{CH}$ ); 2.14 (m, 1H,  $\text{CH}(\text{Me})_2$ ); 0.94 (m, 12H, Me).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 170.8 ( $\text{COCH}$ ); 162.5 ( $\text{COCH}_2$ ); 155.9, 150.3 (NHCO); 141.5 ( $\text{NO}_2\text{C}$ ); 135.6, 128.0, 127.6, 127.5 ( $\text{CH}_2\text{Ph}$ ); 125.4 ( $\text{NO}_2\text{CCH}$ ); 114.1 ( $\text{NO}_2\text{CCHCH}$ ); 66.6 ( $\text{CH}_2\text{Ph}$ ); 66.2 ( $\text{C}_6\text{H}_4\text{OCH}_2$ ); 61.2 ( $\text{CHCH}_2\text{OCO}$ ); 59.9, 56.7 ( $\text{CHNH}$ ); 53.3 ( $\text{CHCH}_2\text{OCO}$ ); 52.3 ( $\text{C}_6\text{H}_4\text{OCH}_2\text{CH}$ ); 31.0, 30.3 ( $\text{CH}(\text{Me})_2$ ); 18.6, 18.4, 17.3, 17.2 (Me). HRMS Calculated for  $\text{C}_{28}\text{H}_{35}\text{N}_3\text{O}_9$  ( $\text{M}^+\text{+H}$ ): 558.24516. Found: 558.24490. FTIR ( $\text{CHCl}_3$ ) 3438; 2969; 1721; 1594; 1517  $\text{cm}^{-1}$ .  $[\alpha]_{\text{D}}^{23} +1.0$  (c 9.0,  $\text{CHCl}_3$ ).

Preparation<sup>2,42</sup> of (E,2*RS*,3*SR*)-Ethyl (2,3-epoxy-5-phenyl)-4-pentenoate (**2.37**) and (E,2*RS*,3*SR*)-Ethyl (2-bromo-3-hydroxy-5-phenyl)-4-pentenoate (**2.38**)

A stirring solution of 2.7 ml (2.7 mmol) of a 1.0 M solution of lithium *bis*(trimethylsilyl)amide in THF (1.2 ml) was cooled to -78 °C under a nitrogen atmosphere. A solution of ethyl bromoacetate (300 mg, 1.8 mmol) in THF (0.8 ml) was added dropwise, the resultant solution was stirred for 10 minutes, and cinnamaldehyde (**2.36**) (211 mg, 1.6 mmol) was added. Stirring was continued for another 20 minutes, at -78 °C, followed by 2 hours at r.t. The reaction was poured into a mixture of iced water and ether. The organic layer was separated, washed (10% HCl and water), dried, and the solvent was evaporated under reduced pressure. Isolation by radial chromatography on silica (80% petroleum ether, 20% ethyl acetate) gave **2.37** (147 mg, 37%) and **2.38** (131 mg, 24%) as colourless oils.

Spectral data for **2.37**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.26-7.40 (m, 5H, ArH); 6.87 (d, 1H, J=16.1 Hz, PhCH); 5.88 (dd, 1H, J=8.3, 16.1 Hz, PhCHCH); 4.28 (dq, 2H, J=3.4, 7.4 Hz, CH<sub>2</sub>); 3.76 (m, 1H, CHCHCO<sub>2</sub>); 3.49 (d, 1H, J=2.0 Hz, CHCO<sub>2</sub>); 1.33 (t, 3H, J=7.3 Hz, Me). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 168.5 (CO<sub>2</sub>); 136.4 (PhCH); 135.5, 128.7, 128.5, 126.6 (Ar); 129.9 (PhCHCH); 61.8 (CH<sub>2</sub>); 58.3 (CHCHCO<sub>2</sub>); 55.1 (CHCO<sub>2</sub>); 14.1 (Me). HRMS Calculated for C<sub>13</sub>H<sub>14</sub>O<sub>3</sub>: 218.09429. Found: 218.09418. FTIR (thin film) 2984; 1747; 1678 cm<sup>-1</sup>.

Spectral data for **2.38**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.26-7.41 (m, 5H, ArH); 6.75 (d, 1H, J=15.7 Hz, PhCH); 6.18 (dd, 1H, J=6.4, 16.1 Hz, PhCHCH); 4.70 (m, 1H, CHOH); 4.35 (d, 1H, J=5.9 Hz, CHBr); 4.25 (dq, 2H, J=1.5, 6.4 Hz, CH<sub>2</sub>); 1.28 (t, 3H, J=7.3 Hz, Me). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 168.4

(CO); 133.9 (PhCH); 135.9, 128.6, 128.3, 126.7 (Ar); 125.8 (PhCHCH); 72.5 (CHOH); 62.4 (CH<sub>2</sub>); 51.5 (CHBr); 13.9 (Me). HRMS Calculated for C<sub>13</sub>H<sub>15</sub><sup>79</sup>BrO<sub>3</sub>: 298.02046. Found: 298.02091. Calculated for C<sub>13</sub>H<sub>15</sub><sup>81</sup>BrO<sub>3</sub>: 300.01841. Found: 300.01837. FTIR (thin film) 3459(br); 2982; 2360; 1733 cm<sup>-1</sup>.

Preparation<sup>2.45</sup> of (*E*,2*SR*,3*SR*)-Ethyl (2,3-epoxy-5-phenyl)-4-pentenoate (**2.39**)

A stirring solution of 131 mg (0.45 mmol) of **2.38** in THF (8.0 ml) was cooled to 0 °C under a nitrogen atmosphere and potassium *tert*-butoxide (55 mg, 0.45 mmol), suspended in THF (3.0 ml), was added dropwise while maintaining the temperature below 15 °C. The resultant solution was stirred for 10 minutes and the reaction was quenched by the addition of a 3% solution of ammonium chloride. The organic layer was separated and the aqueous layer was extracted with chloroform (5x). The combined organic extracts were dried (MgSO<sub>4</sub>) and the solvent was evaporated under reduced pressure. Isolation by radial chromatography on silica (90% petroleum ether, 10% ethyl acetate) gave **2.39** as a colourless oil (102 mg, 72%).

Spectral data for **2.39**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.28-7.39 (m, 5H, ArH); 6.91 (d, 1H, J=16.2 Hz, PhCH); 6.15 (dd, 1H, J=8.3, 16.1 Hz, PhCHCH); 4.29 (dq, 2H, J=3.4, 7.4 Hz, CH<sub>2</sub>); 3.78 (m, 2H, CH(epoxide)); 1.30 (t, 3H, J=6.9 Hz, Me). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 167.8 (CO<sub>2</sub>); 137.9 (PhCH); 135.7, 128.7, 128.5, 126.7 (Ar); 121.5 (PhCHCH); 61.6 (CH<sub>2</sub>); 57.9, 54.6 (C(epoxide)); 14.2 (Me). HRMS Calculated for C<sub>13</sub>H<sub>14</sub>O<sub>3</sub>: 218.09429. Found: 218.09454. FTIR (thin film) 2930; 2860; 1732; 1580 cm<sup>-1</sup>.

Preparation<sup>2,43</sup> of (2*RS*,3*SR*)-Ethyl 2,3-epoxy-(5-phenyl)pentanoate (**2.41**) and (*R,S*)-Ethyl (2-hydroxy-5-phenyl)pentanoate (**2.42**)

To a solution of 90 mg (0.42 mmol) of **2.37** in ethyl acetate (20 ml), was added 10% palladium on carbon (26 mg) and the resultant solution was stirred under a hydrogen atmosphere for 2 hours. The solution was then filtered and the solvent was evaporated under reduced pressure. Isolation by radial chromatography on silica (80% petroleum ether, 20% ethyl acetate) gave **2.41** (18 mg, 19%) and **2.42** (27 mg, 29%) as colourless oils.

Spectral data for **2.41**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.18-7.33 (m, 5H, ArH); 4.22 (dq, 2H, J=2.0, 7.3 Hz, OCH<sub>2</sub>); 3.20 (m, 2H, CH(epoxide)); 2.81 (m, 2H, PhCH<sub>2</sub>); 1.92 (m, 2H, PhCH<sub>2</sub>CH<sub>2</sub>); 1.29 (t, 3H, J=6.8 Hz, Me). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 169.1 (CO<sub>2</sub>); 140.5, 128.5, 128.4, 126.2 (Ar); 61.5 (OCH<sub>2</sub>); 57.8, 53.2 (C(epoxide)); 33.3 (PhCH<sub>2</sub>); 31.9 (PhCH<sub>2</sub>CH<sub>2</sub>); 14.1 (Me). HRMS Calculated for C<sub>13</sub>H<sub>16</sub>O<sub>3</sub>: 220.10994. Found: 220.11040. FTIR (thin film) 3454; 3026; 2932; 2864; 2343; 1732; 1605 cm<sup>-1</sup>.

Spectral data for **2.42**:

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.17-7.31 (m, 5H, ArH); 4.23 (q, 2H, J=7.3 Hz, OCH<sub>2</sub>); 4.19 (t, 1H, J=4.9 Hz, CHOH); 2.90 (m(br), 1H, OH); 2.65 (m, 2H, PhCH<sub>2</sub>); 1.66-1.87 (m, 4H, (CH<sub>2</sub>)<sub>2</sub>CHOH); 1.28 (t, 3H, J=6.8 Hz, Me). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 175.2 (CO<sub>2</sub>); 141.9, 128.4, 128.3, 125.8 (Ar); 70.3 (CHOH); 61.6 (OCH<sub>2</sub>); 35.5 (PhCH<sub>2</sub>); 33.9, 26.5 ((CH<sub>2</sub>)<sub>2</sub>CHOH); 14.2 (Me). HRMS Calculated for C<sub>13</sub>H<sub>18</sub>O<sub>3</sub>: 222.12559. Found: 222.12595. FTIR (thin film) 3028; 2984; 2930; 2862; 1749; 1605 cm<sup>-1</sup>.

## References

- 2.1. Huff, J. *J. Med. Chem.* **1991**, 34 (8), 2305.
- 2.2. Meek, T. *Enzyme Inhib.* **1992**, 6, 65.
- 2.3. Trova, M. Babine, R. Byrn, R. Casscles, W. Hastings, R. Hsu, G. Jirousek, M. Johnson, B. Kerwar, S. Schow, S. Wissner, A. Zhang, N. Wick, M. *Bioorg. Med. Chem. Lett.* **1993**, 3 (8), 1595.
- 2.4. Anderson, A. *New Scientist.* **1997**, 154 (2067), 3.
- 2.5. Metcalf, B. Debouck, C. Dreyer, G. Lambert, D. Tomaszek, T. Meek, T. Petteway, S. In: *New Leads and Targets in Drug Research, Alfred Benzon Symposium* 33. Editors: Krogsgaard-Larsen, P. Brogger Christensen, S. Kofod, H. Munksgaard, Copenhagen. **1992**, 98.
- 2.6. Johnson, M. Allaudeen, H. Sarver, N. *TiPS.* **1989**, 10, 305.
- 2.7. Franciskovich, J. Houseman, K. Mueller, R. Chmielewski, J. *Bioorg. Med. Chem. Lett.* **1993**, 3 (4), 765.
- 2.8. Navia, M. Fitzgerald, P. McKeever, B. Leu, C. Heimbach, J. Herber, W. Sigal, I. Darke, P. Springer, J. *Nature.* **1989**, 337, 615.
- 2.9. West, M. Fairlie, D. *TiPS.* **1995**, 16, 67.
- 2.10. Wlodawer, A. Erickson, J. *Annu. Rev. Biochem.* **1993**, 62, 543.
- 2.11. Sakurai, M. Sugano, M. Handa, H. Komai, T. Yagi, R. Nishigaki, T. Yabe, Y. *Chem. Pharm. Bull.* **1993**, 41 (8), 1369.
- 2.12. Schechter, I. Berger, A. *Biochem. Biophys. Res. Comm.* **1967**, 27, 157.
- 2.13. Rodriguez, E. Angeles, T. Meek. T. *Biochem.* **1993**, 32, 12380.

- 2.14. Kempf, D. Norbeck, D. Codacovi, L. Wang, X. Kohlbrenner, W. Wideburg, N. Paul, D. Knigge, M. Vasavanonda, S. Craig-Kennard, A. Saldivar, A. Rosenbrook, W. Clement, J. Plattner, J. Erickson, J. *J. Med. Chem.* **1990**, *33* (10), 2687.
- 2.15. Erickson, J. Neidhart, D. VanDrie, J. Kempf, D. Wang, X. Norbeck, D. Plattner, J. Rittenhouse, J. Turon, M. Wideburg, N. Kohlbrenner, W. Simmer, R. Helfrich, R. Paul, D. Knigge, M. *Science*. **1990**, *249*, 527.
- 2.16. Brinkworth, R. Woon, T. Fairlie, D. *Biochem. Biophys. Res. Comm.* **1991**, *176* (1), 241.
- 2.17. Moelling, K. Schulze, T. Knoop, M. Kay, J. Jupp, R. Nicolaou, G. Pearl, L. *Febs Letters*. **1990**, *261*, (2), 373.
- 2.18. Blumenstein, J. Copeland, T. Oroszlan, S. Michejda, C. *Biochem. Biophys. Res. Comm.* **1989**, *163* (2), 980.
- 2.19. Rose, R. Rose, J. Salto, R. Craik, C. Stroud, R. *Biochem.* **1993**, *32*, 12498.
- 2.20. Grant, S. Moore, M. Fakhoury, S. Tomaszek, T. Meek, T. *Bioorg. Med. Chem. Lett.* **1992**, *2* (11), 1441.
- 2.21. Lee, C. Choy, N. Park, C. Choi, H. Son, Y. Kim, S. Ok, J. Yoon, H. Kim, S. *Bioorg. Med. Chem. Lett.* **1996**, *6* (6), 589.
- 2.22. Yu, Z. Caldera, P. McPhee, F. De Voss, J. Jones, P. Burlingame, A. Kuntz, I. Craik, C. Ortiz de Montellano, P. *J. Am. Chem. Soc.* **1996**, *118*, 5846.
- 2.23. Park, C. Koh, J. Son, Y. Choi, H. Lee, C. Choy, N. Moon, K. Jung, W. Kim, S. Yoon, H. *Bioorg. Med. Chem. Lett.* **1995**, *5* (16), 1843.
- 2.24. Windridge, G. Jorgensen, E. *J. Am. Chem. Soc.* **1971**, *93* (23), 6318.
- 2.25. Kirshenbaum, K. Sharpless, K. *J. Org. Chem.* **1985**, *50* (11), 1979.
- 2.26. Bone, R. Vacca, J. Anderson, P. Holloway, K. *J. Am. Chem. Soc.* **1991**, *113*, 9382.

- 2.27. Gour-Salin, B. Lachance, P. Plouffe, C. Storer, A. Menard, R. *J. Med. Chem.* **1993**, *36*, 720.
- 2.28. Blatt, A. *Organic Synthesis Collective Volume 2*. John Wiley & Sons, Inc., New York. **1943**, 28.
- 2.29. Kupryszewski, G. Sokolowska, T. *Acta. Biochim. Polon.* **1957**, *4*, 85.
- 2.30. Tamai, M. Yokoo, C. Murata, M. Oguma, K. Sota, K. Sato, E. Kanaoka, Y. *Chem. Pharm. Bull.* **1987**, *35* (3), 1098.
- 2.31. Bremser, W. Ernst, L. Fachinger, W. Gerhards, R. Hardt, A. Lewis, P. *Carbon-13 NMR Spectral Data*. 4th. ed. VCH Verlagsgesellschaft mbH, Weinheim, **1987**.
- 2.32. Pouchet, C. *The Aldrich Library of NMR Spectra*. 2nd. ed. Aldrich Chemical Company, Inc., Wisconsin. **1983**, *1*, 568C.
- 2.33. Pouchet, C. *The Aldrich Library of NMR Spectra*. 2nd. ed. Aldrich Chemical Company, Inc., Wisconsin. **1983**, *1*, 568B.
- 2.34. Ando, K. *Tetrahedron Lett.* **1995**, *36* (23), 4105.
- 2.35. Lopez Aparicio, F. Garcia Mendoza, P. Zorrilla Benitez, F. Santoyo Gonzalez, F. *Anales De Quimica.* **1984**, *80*, 83.
- 2.36. Marshall, J. Trometer, J. Cleary, D. *Tetrahedron.* **1989**, *45* (2), 391.
- 2.37. Katsuki, T. Martin, V. In: *Organic Reactions*. Editor: Paquette, L. John Wiley and Sons, Inc., New York. **1996**, *48*, 1 and references therein.
- 2.38. Carlsen, P. Katsuki, T. Martin, V. Sharpless, K. *J. Org. Chem.* **1981**, *46* (19), 3936.
- 2.39. Jones, J. *Amino Acid and Peptide Synthesis*. Oxford University Press, Oxford. **1992**, 33.
- 2.40. Gao, Y. Hanson, R. Klunder, J. Ko, S. Masamune, H. Sharpless, K. *J. Am. Chem. Soc.* **1987**, *109*, 5765.

- 2.41. Mitsunobu, O. *Synthesis*. **1981**, January, 1.
- 2.42. Borch, R. *Tetrahedron Lett.* **1972**, 36, 3761.
- 2.43. Furniss, B. Hannaford, A. Smith, P. *Vogel's Textbook of Practical Organic Chemistry*. 5th. ed. John Wiley & Sons, Inc., New York. **1989**, 472.
- 2.44. Tarbell, D. Carman, R. Chapman, D. Cremer, S. Cross, A. Huffman, K. Kunstmann, M. McCorkindale, N. McNally, J. Rosowsky, A. Varino, F. West, R. *J. Am. Chem. Soc.* **1961**, 83 (3), 3096.
- 2.45. Truce, W. Christensen, L. *J. Org. Chem.* **1971**, 36 (17), 2538.
- 2.46. Roux-Schmitt, M. Seyden-Penne, J. *Tetrahedron*. **1972**, 28, 4965.
- 2.47. Williams, D. Fleming, I. *Spectroscopic Methods in Organic Chemistry*. 2nd. ed. McGraw-Hill Book Company (U.K.) Ltd., England. **1973**.
- 2.48. Silverstein, R. Bassler, G. Morrill, T. *Spectrometric Identification of Organic Compounds*. 4th. ed. John Wiley & Sons, Inc., New York. **1981**, 189.
- 2.49. Bergman, D. Alewood, D. Alewood, P. Andrews, J. Brinkworth, R. Englebretsen, D. Kent, S. *Lett. Pept. Sci.* **1995**, 2, 99.
- 2.50. Bodanszky, M. Bodanszky, A. *The Practice of Peptide Synthesis*. Springer-Verlag, New York. **1984**, 145.

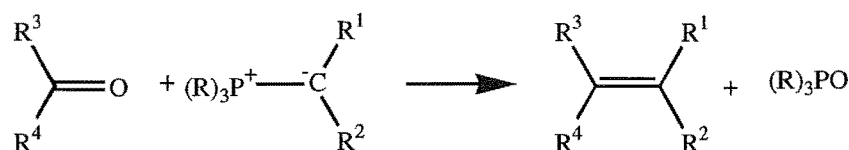


## Chapter 3. Stereochemistry of the “Horner-Wadsworth-Emmons” Reaction

### Introduction

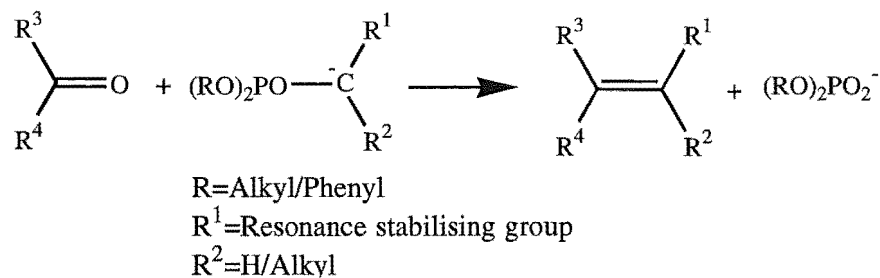
The controlled introduction of carbon-carbon double bonds is of considerable interest in organic chemistry.<sup>3.1, 3.2</sup> The development of the Wittig reaction, over 30 years ago, provided the first general and effective method of carbon-carbon double bond formation.<sup>3.2</sup> The conventional Wittig reaction involves the reaction of a phosphorous ylide with an aldehyde or ketone to form an olefin and a phosphine oxide (Scheme 3.1).<sup>3.1, 3.2</sup> This reaction and subsequent modifications rapidly became widely used, changing the face of olefin synthesis for all time.<sup>3.2</sup>

Scheme 3.1



Almost a decade after the disclosure of the Wittig reaction, a complementary reaction was developed by Horner *et al.*<sup>3.1</sup> and by Wadsworth and Emmons.<sup>3.1</sup> This reaction is variously called the “modified Wittig” reaction, the “Wittig-Horner” reaction, the “Horner-Emmons” reaction, and the “Horner-Wadsworth-Emmons” reaction. This thesis refers to it as the “Horner-Wadsworth-Emmons” (HWE) reaction. The HWE reaction employs a resonance stabilised phosphonate carbanion which undergoes reaction with an aldehyde or ketone, as shown in Scheme 3.2, to give an olefin and a phosphate ion.<sup>3.1</sup>

Scheme 3.2



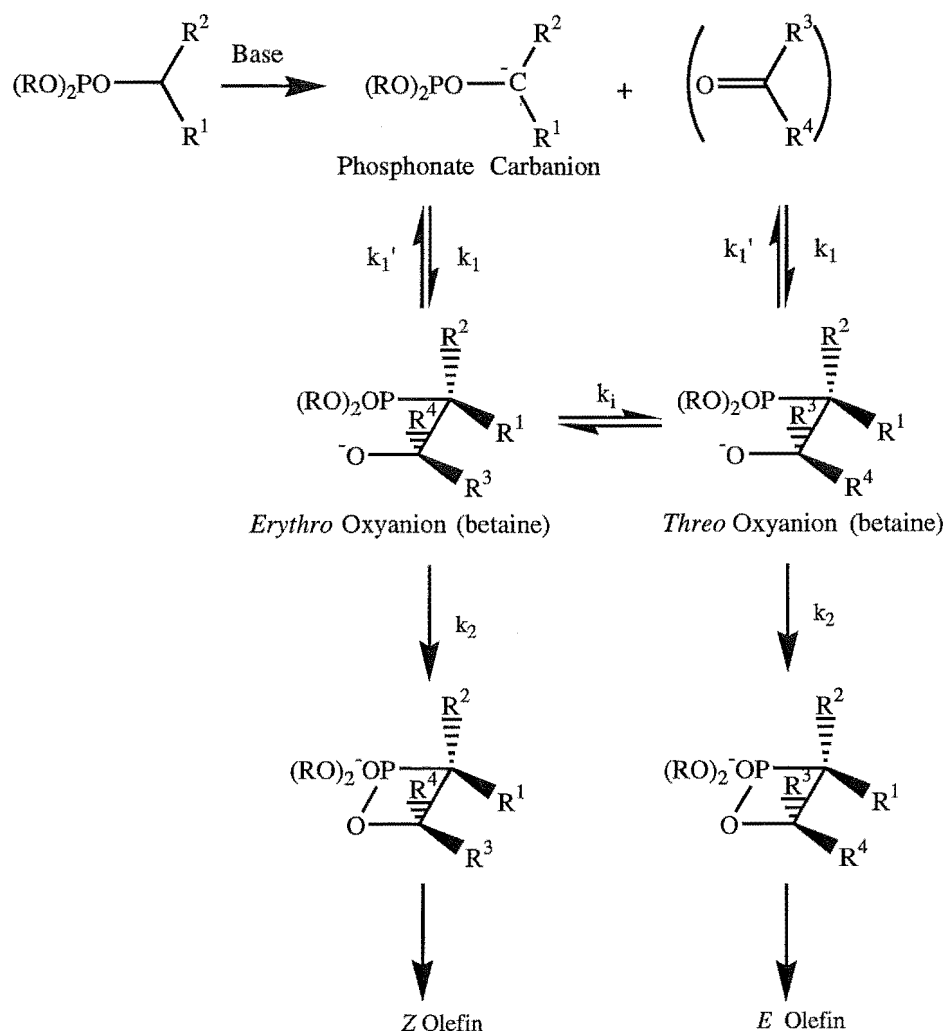
The HWE reaction possesses a number of advantages over the Wittig reaction.<sup>3.1</sup> Most importantly, phosphonate carbanions are more nucleophilic than phosphonium ylides. This is attributed to a decreased stabilisation of the negative charge by valence shell expansion of the phosphorous atom in the phosphonate carbanion.<sup>3.1</sup> Therefore, phosphonate carbanions react with a wider variety of aldehydes and ketones and under milder conditions. Further, this enhanced reactivity allows for alkylation of the carbon, alpha to the phosphorous atom. On a practical note, phosphonates are readily prepared by the Arbuzov reaction<sup>3.3</sup> and the phosphate ion by-product is water soluble, allowing for easy removal from the reaction mixture.

### Part 1. Mechanism of the HWE Reaction

The widely accepted mechanism of the HWE reaction, presented in Scheme 3.3, is analogous to that of the conventional Wittig reaction.<sup>3.1, 3.2, 3.4, 3.5</sup> The first step involves the base promoted formation of the phosphonate carbanion (see Scheme 3.3), stabilised by the electron withdrawing group ( $\text{R}^1$ ) and the phosphoryl group. Step 2 is a reversible aldol condensation, giving two diastereoisomeric oxyanions (betaines) (see Scheme 3.3). The irreversible decomposition of these betaines to olefins is thought to involve *syn* elimination via a four membered cyclic transition state which may have some double bond character. Thus, the *erythro* betaine leads, stereospecifically, to the *Z* olefin and the

*threo* betaine gives the *E* olefin (see Scheme 3.3). There is no appreciable interconversion of the *E* and *Z* olefins under the reaction conditions.<sup>3.1</sup> Kinetic studies, performed by Larsen and Aksnes, found the reaction was first order with respect to the aldehyde, the base, and the phosphonate, and third order overall.<sup>3.2</sup> The rate limiting step being the initial condensation of the aldehyde with the phosphonate.<sup>3.2</sup>

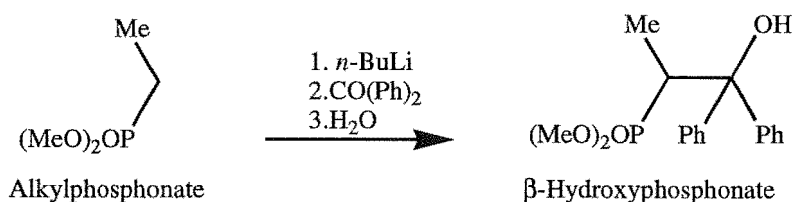
Scheme 3.3



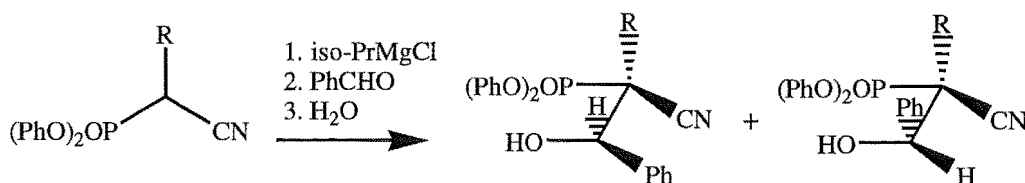
Although there has been no direct observation of the intermediates, the isolation of  $\beta$ -hydroxyphosphonates (protonated oxyanions) has provided strong supporting evidence for the intermediacy of an oxyanion.<sup>3.5</sup> For example, Scheme 3.4 shows that an alkyl phosphonate which has a methyl group on the carbon alpha to the phosphorous (rather

than an electron withdrawing substituent), yields a  $\beta$ -hydroxyphosphonate upon treatment with base, followed by reaction with benzophenone and subsequent hydrolysis.<sup>3,5</sup> The  $\beta$ -hydroxyphosphonate does not undergo elimination to form an olefin even under basic conditions. A phosphonate with an electron withdrawing substituent on the carbon alpha to the phosphorous generally leads to spontaneous decomposition to olefins (see Scheme 3.2). This suggests that the transition state for elimination has a considerable build up of negative charge on the carbon alpha to the phosphorous atom.<sup>3,4,3,5</sup> However, the use of a magnesium cation results in a more stable intermediate (since magnesium salts have more covalent character) and Scheme 3.5 illustrates the formation of a pair of resonance stabilised  $\beta$ -hydroxyphosphonates.<sup>3,5</sup> Thermal decomposition of these isolated intermediates was shown to be highly stereospecific, occurring in a *syn* fashion.<sup>3,5</sup>

Scheme 3.4



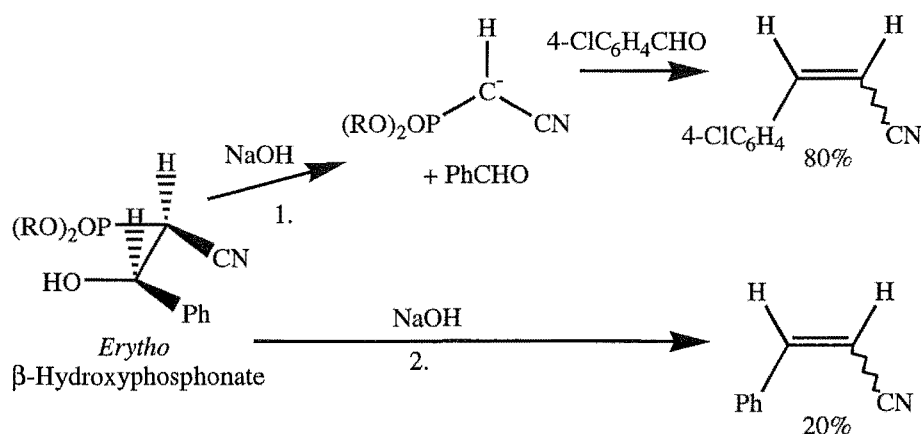
Scheme 3.5



A study by Seyden-Penne *et al.* demonstrated that the intermediate betaines (see Scheme 3.3) are capable of both retroaldolisation and interconversion.<sup>3,6</sup> The *erythro*  $\beta$ -hydroxyphosphonate, shown in Scheme 3.6, was prepared, isolated, and reacted with 4-chlorobenzaldehyde (two equivalents) and sodium hydroxide ( $\text{NaOH}$ ) in

tetrahydrofuran (THF) (analogous studies were performed on the equivalent *threo*  $\beta$ -hydroxyphosphonate).<sup>3,6</sup> The product mixture contained 80% 4-chlorocinnamitrile, demonstrating that the  $\beta$ -hydroxyphosphonate was capable of retroaldolisation (via pathway 1, Scheme 3.6).<sup>3,6</sup> Further, the 20% cinnamitrile formed (via pathway 2, Scheme 3.6) had a similar stereochemical ratio as the direct reaction between the phosphonate carbanion and benzaldehyde. This demonstrates that the *erythro* intermediate is capable of direct epimerisation to the *threo* form (any phosphonate carbanion formed by retroaldolisation would be trapped by the more reactive 4-chlorobenzaldehyde).<sup>3,6</sup> Note that a hydrogen on the carbon alpha to the phosphorous atom is required for epimerisation.<sup>3,5, 3,6</sup>

Scheme 3.6



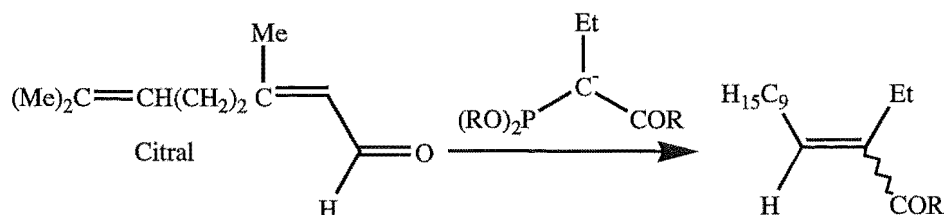
## Part 2. Stereochemistry of the Olefin Products

Initial studies of HWE olefin formation reported exclusively *E* stereochemistry.<sup>3.1, 3.4, 3.5</sup>

However, reports of *Z* olefins soon began to appear.<sup>3.1, 3.4, 3.5</sup> For example, an early study<sup>3.5</sup> showed that small, but appreciable, quantities of the *Z* isomer were obtained

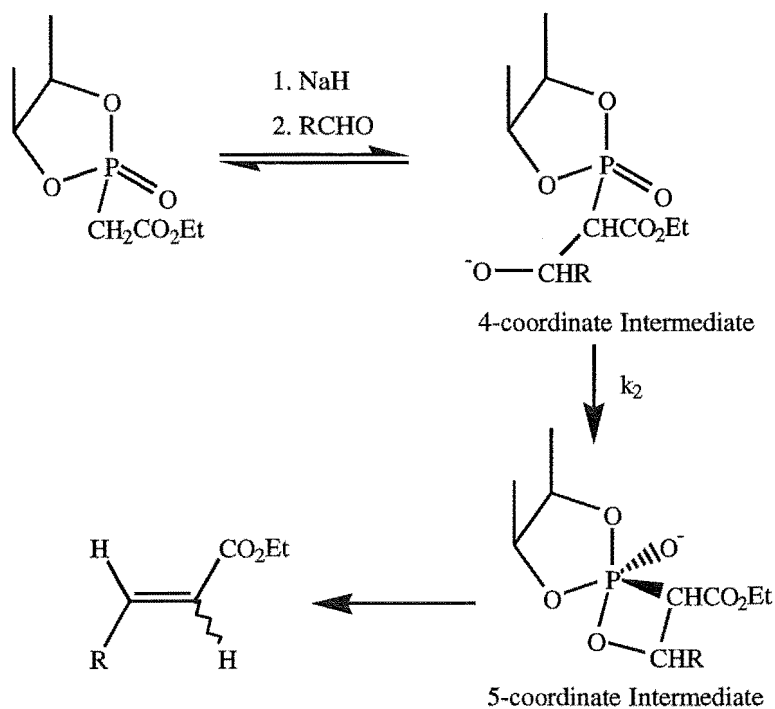
when citral was reacted with a phosphonate anion, where the anion has an ethyl group on the carbon, alpha to the phosphorous atom (Scheme 3.7).

Scheme 3.7



It is generally accepted that the stereochemistry of the olefin formed depends upon the relative rates of formation ( $k_1$ , Scheme 3.3) and of decomposition ( $k_1'/k_2$ , Scheme 3.3— $k_1'/k_2$  is often referred to as the “reversibility factor”<sup>3,7</sup>) of both the *erythro* and *threo* betaines.<sup>3,4, 3.7, 3.8</sup> It is well established that the “reversibility factor” is of prime importance in determining the stereochemical outcome of the reaction.<sup>3,4, 3.7, 3.8, 3.9</sup> The *Z* olefin is favoured when the “reversibility factor” decreases. For example, the reaction of cyclic phosphonates with an aldehyde (Scheme 3.8), led to a high proportion of the *Z* olefin in every case studied.<sup>3,10</sup> This was attributed to a decrease in the “reversibility factor” due to an increase in the rate of decomposition ( $k_2$ ), promoted by the release of ring strain (upon passing from a four co-ordinate to a five co-ordinate intermediate).<sup>3,10</sup> Conversely, the *E* olefin is favoured when  $k_1'$  (Scheme 3.3) is large, for example in the case of aromatic aldehydes.<sup>3,8</sup> It is generally accepted that the more stable *E* olefin forms as a result of thermodynamic control.<sup>3,8</sup>

Scheme 3.8



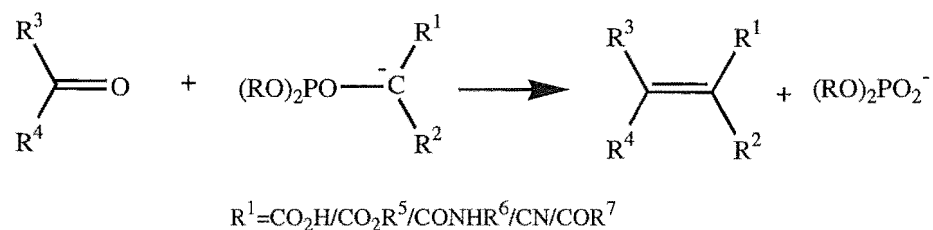
A number of factors, including the reaction conditions and nature of the reactants, have been shown to affect the stereochemical outcome of the reaction.<sup>3.1, 3.2, 3.4, 3.5, 3.7, 3.8, 3.9, 3.10</sup> For example, studies on the influence of the metal cation (supplied by the base) have shown that lithium offers the best *E* selectivity.<sup>3.5, 3.7</sup> This has been attributed to a high degree of association between lithium and the intermediate betaines, resulting in a decrease in the rate of decomposition to product (and a consequent increase in the “reversibility factor”).<sup>3.7</sup> Further, the reaction temperature has considerable influence on the stereochemical outcome of the reaction.<sup>3.7</sup> A decrease in temperature leads to an increase in the amount of *Z* product, attributed to a decrease in the reversibility of the reaction at low temperatures.<sup>3.7</sup>

The effect on the HWE reaction of the nature of the aldehyde and of modifications to the phosphoryl reagent are the subject of this chapter. The effect of the reaction temperature and the solvent are also discussed briefly.

### Part 3. Applications of the HWE Reaction in Synthesis

The HWE reaction has been employed to extend the scope of the traditional Wittig reaction.<sup>3,4</sup> The stereospecificity and experimental simplicity of the HWE reaction have made it a method of choice for the synthesis of many alkenes.<sup>3,5</sup> As discussed above, the phosphonate carbanion requires the presence of an electron withdrawing group at R<sup>1</sup> (see Scheme 3.2). Therefore, the synthesis is generally limited to alkenes containing at least one activating group alpha to the double bond.<sup>3,4</sup> Modifications to the structure of the phosphonate allows the preparation of  $\alpha,\beta$ -unsaturated products containing a wide variety of functional groups. For example, an appropriate substituent at R<sup>1</sup> of the phosphonate carbanion enables the synthesis of  $\alpha,\beta$ -unsaturated acids, esters, amides, and nitriles in excellent yields (see Scheme 3.2).<sup>3,4</sup> The superior reactivity of the HWE reaction, over the Wittig reaction, allows the synthesis of  $\alpha,\beta$ -unsaturated ketones, although poor yields have been noted in some cases.<sup>3,4</sup>

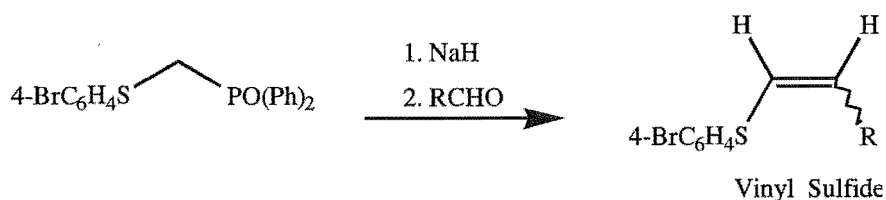
Scheme 3.2





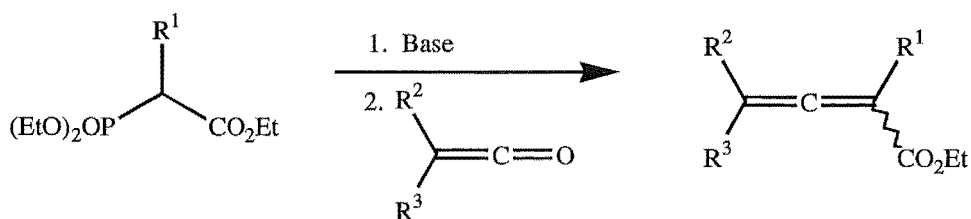
Various sulfur compounds may also be prepared in high yields.<sup>3,4,3.5</sup> For example, Scheme 3.9 shows the synthesis of a vinyl sulfide from a modified phosphonate and an aldehyde.<sup>3.5</sup>

Scheme 3.9

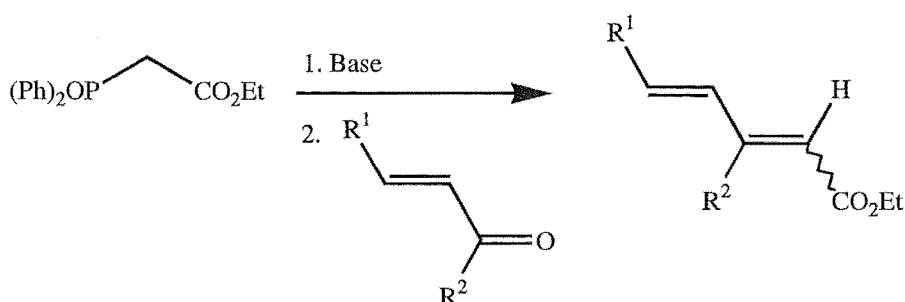


Modification of the carbonyl co-reactant widens the scope of the HWE reaction. For example, the synthesis of allenes from ketenes, shown in Scheme 3.10, is preferred to the Wittig route due to the milder reaction conditions required.<sup>3.4</sup> Further, dienes may be prepared from unsaturated carbonyl compounds, as shown in Scheme 3.11.<sup>3.4</sup> Although competing reactions can sometimes interfere, the yield is often better than that from the corresponding Wittig reaction.<sup>3.4</sup>

Scheme 3.10



Scheme 3.11





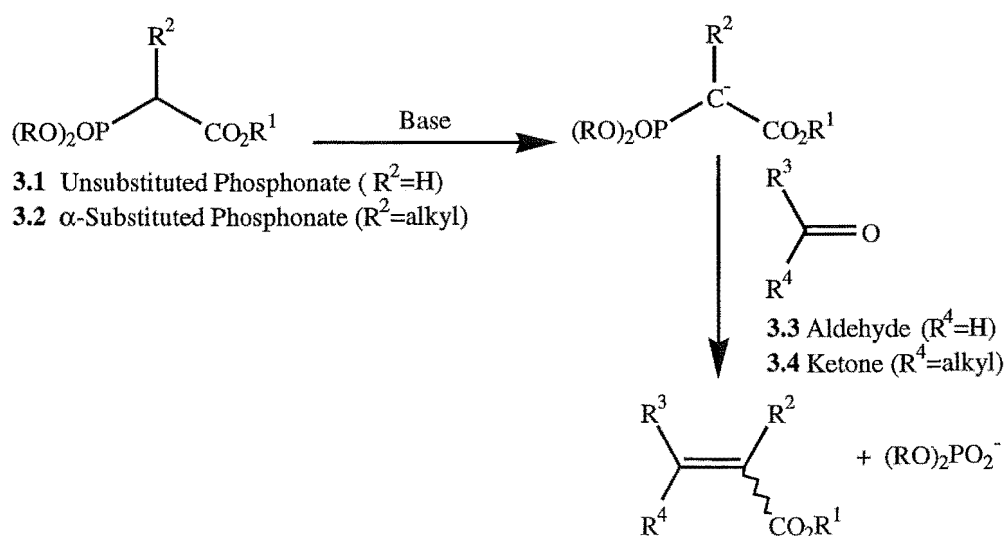
## Results and Discussion

### Part 1. Effect of the Aldehyde on the HWE Reaction

In the HWE reaction (Scheme 3.13), between a phosphonate (**3.1/3.2**) and an aldehyde (**3.3**) or ketone (**3.4**), it has been noted that the nature of the carbonyl substituent(s) ( $R^3/R^4$ ) can effect the *E:Z* stereochemistry of the product.<sup>3.2, 3.4, 3.5, 3.7, 3.11, 3.12, 3.13</sup>

Heathcock and Thompson,<sup>3.7</sup> on studying the HWE reaction with various aldehydes, stated that, of all the reaction variables, the nature of the alkyl substituent of the aldehyde had the greatest effect on the stereochemistry of the product. However, the emergence of clear trends has been elusive. Our investigations have sought to establish a relationship between the nature of the aldehyde (**3.3**, Scheme 3.13) and the stereochemical outcome of the reaction and to compare this with literature investigations.

Scheme 3.13

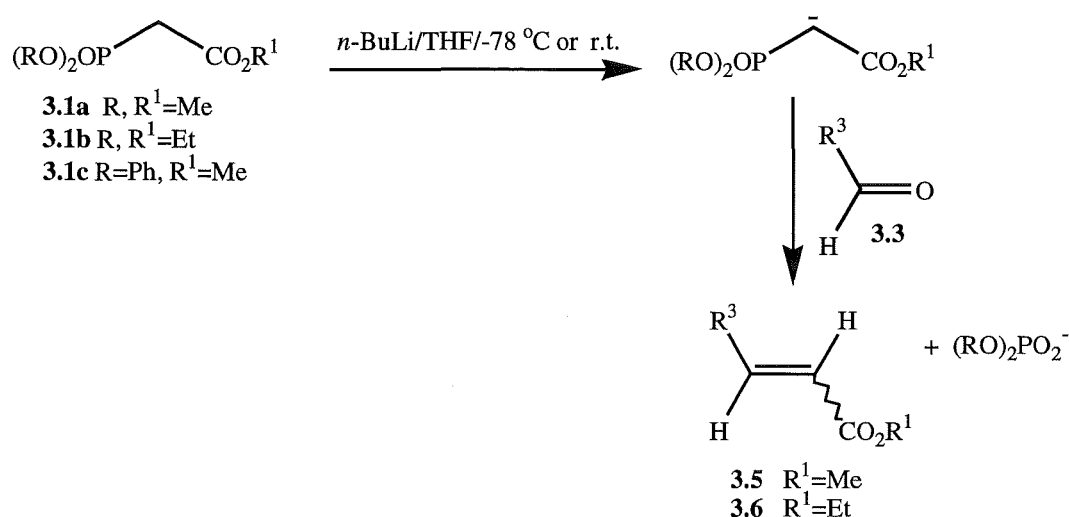


Three classes of aldehydes (**3.3**) were studied, where  $R^3$  is either conjugated, unbranched aliphatic, or alpha-branched aliphatic (see Table 3.1 for numbering of the aldehydes (**3.3**)). Each class gave characteristic results. The aldehydes (**3.3**) were reacted with the

corresponding anions of the phosphonates (**3.1a**, **3.1b**, or **3.1c**) as shown in Scheme 3.14.

The reactions were performed as described by Heathcock and Thompson,<sup>3,7</sup> using *n*-butyllithium (*n*-BuLi) as the base and tetrahydrofuran (THF) as the solvent, at either room temperature (r.t.) or at -78 °C. The *E:Z* isomer ratio of the olefin products was determined by <sup>1</sup>H NMR analysis of the crude reaction mixture.

Scheme 3.14

Table 3.1. Numbering of the Aldehydes (**3.3**)

Aldehyde (R <sup>3</sup> CHO)	R <sup>3</sup>
<b>3.3a</b>	Ph
<b>3.3b</b>	PhCH=CH
<b>3.3c</b>	Me
<b>3.3d</b>	Et
<b>3.3e</b>	Me(CH <sub>2</sub> ) <sub>5</sub>
<b>3.3f</b>	Me(CH <sub>2</sub> ) <sub>9</sub>
<b>3.3g</b>	(Me) <sub>2</sub> CH
<b>3.3h</b>	(Et) <sub>2</sub> CH
<b>3.3i</b>	cyclohexyl
<b>3.3j</b>	Me(CH <sub>2</sub> ) <sub>8</sub> (Me)CH
<b>2.25a</b>	PhCH <sub>2</sub> CH <sub>2</sub>
<b>2.25b</b>	4-nitroC <sub>6</sub> H <sub>4</sub> OCH <sub>2</sub>

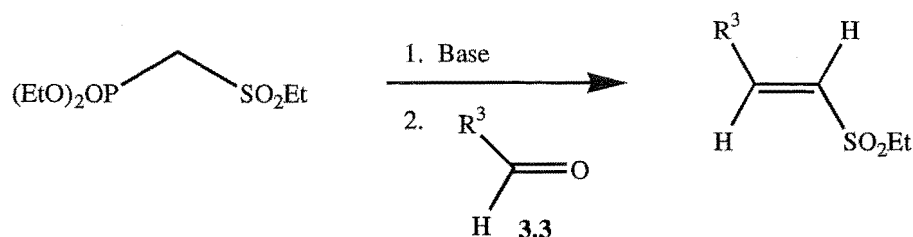
The effect of a conjugated aldehyde (**3.3**, where  $R^3$  is unsaturated or aromatic) on the stereochemical outcome of the HWE reaction was investigated using benzaldehyde (**3.3a**) and cinnamaldehyde (**3.3b**). As shown in Table 3.2, the aldehydes (**3.3a** and **3.3b**) were reacted with the anion of trimethyl phosphonoacetate (**3.1a**), at  $-78\text{ }^{\circ}\text{C}$ , in THF, using *n*-BuLi to generate the anion. The results, presented in Table 3.2, demonstrated a very strong preference for the formation of the *E* olefin.

Table 3.2. Product ratios obtained with Conjugated Aldehydes (**3.3**)

$(\text{MeO})_2\text{POCH}_2\text{CO}_2\text{Me}$ ( <b>3.1a</b> ) $\xrightarrow[2. \text{R}^3\text{CHO} (\textbf{3.3})]{1. n\text{-BuLi/THF/-78 }^{\circ}\text{C}}$ $\text{R}^3\text{HC=CHCO}_2\text{Me}$ ( <b>3.5</b> )	
Aldehyde ( <b>3.3</b> )	<i>E</i> : <i>Z</i> ratio
<b>3.3a</b> ( $\text{R}^3=\text{Ph}$ )	>19:1
<b>3.3b</b> ( $\text{R}^3=\text{PhCH=CH}$ )	>19:1

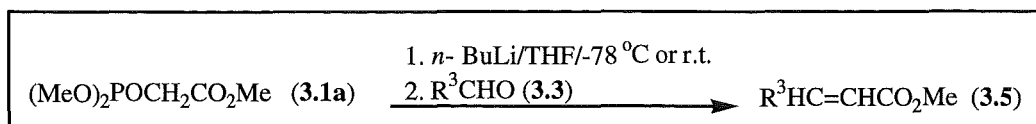
A predominance of the *E* olefin in the HWE reaction of conjugated aldehydes has been noted in other systems.<sup>3,4, 3.12, 3.14</sup> For example, Popoft *et al.*<sup>3,14</sup> studied the reaction of various aldehydes (**3.3**, where  $R^3$  has the ability to conjugate) with the phosphonate  $(\text{EtO})_2\text{POCH}_2\text{SO}_2\text{Et}$ , as shown in Scheme 3.15. Their investigation showed that the *E* isomer predominated when  $R^3$  is conjugated.<sup>3,14</sup> It was suggested that the *E* isomer was favoured because of the increased steric bulk of  $R^3$  when it is conjugated.<sup>3,14</sup> However, our work on aliphatic aldehydes (see later) suggested that the steric effect of  $R^3$  is not straightforward and that bulkier groups do not necessarily promote formation of the *E* isomer. Further, a predominance of the *E* isomer has also been noted<sup>3,12</sup> for the reaction between the alpha-substituted phosphonate (**3.2**  $\text{R}=\text{Et}$ ,  $\text{R}^1, \text{R}^2=\text{Me}$ ) and alpha, beta-unsaturated aldehydes (**3.3**) (see Scheme 3.13 for a general scheme).

Scheme 3.15



Therefore, our findings that the conjugated aldehydes, benzaldehyde (**3.3a**) and cinnamaldehyde (**3.3b**), reacted with trimethyl phosphonoacetate (**3.1a**) to give a predominance of the *E* olefin (Table 3.2) was consistent with literature reports on similar systems.<sup>3,4, 3.12, 3.14</sup> In every case presented in this chapter, reaction with benzaldehyde (**3.3a**) resulted in a larger proportion of the *E* product than any of the aliphatic aldehydes studied, under the same reaction conditions. It was not considered necessary to investigate this area further.

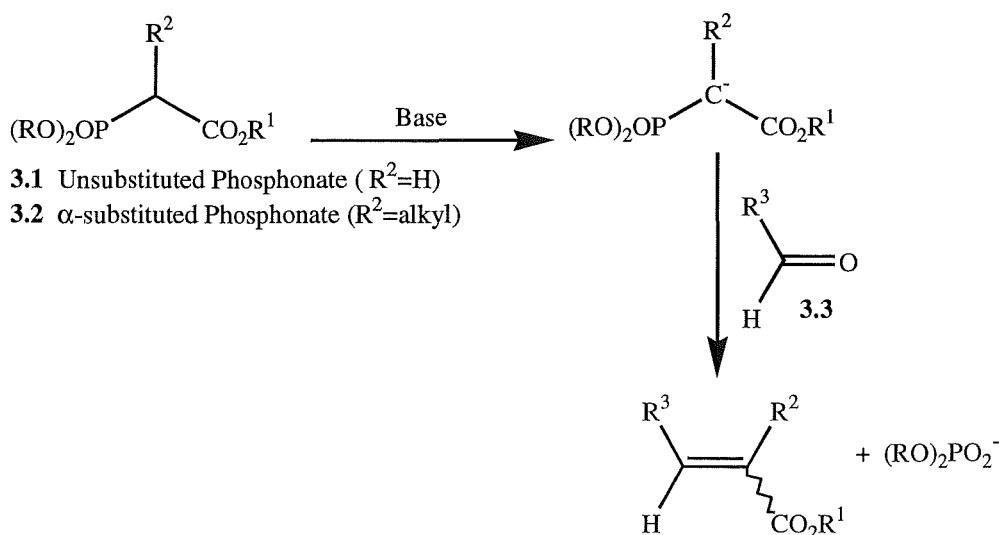
The effect of the alkyl group of a non-conjugated aldehyde (**3.3**, where  $\text{R}^3$  is a straight chain) on the stereochemical outcome of the HWE reaction was investigated using the four aldehydes, acetaldehyde (**3.3c**), propionaldehyde (**3.3d**), heptaldehyde (**3.3e**), and undecanal (**3.3f**). As shown in Table 3.3, the aldehydes were reacted with the anion of trimethyl phosphonoacetate (**3.1a**), at  $-78\text{ }^\circ\text{C}$  and at r.t., in THF, using *n*-BuLi to generate the anion. An examination of the results presented in Table 3.3 reveals that in all cases the *E* olefin predominated, but that in most cases a significant amount of the *Z* isomer was formed. The proportion of the *Z* isomer formed, both at r.t. and  $-78\text{ }^\circ\text{C}$ , appeared to vary with the length of the carbon chain at  $\text{R}^3$ . The pattern observed for the four aliphatic aldehydes (**3.3c-3.3f**, Table 3.3) investigated, indicated that the longer the carbon chain at  $\text{R}^3$ , the greater the proportion of *Z* product.

Table 3.3. Product ratios obtained with Unbranched Aliphatic Aldehydes (**3.3c-f**)

Aldehyde ( <b>3.3</b> )	<i>E:Z</i> Product Ratio	
	at -78 °C	at r.t.
<b>3.3c</b> ( $\text{R}^3=\text{Me}$ )	8.0:1	11:1
<b>3.3d</b> ( $\text{R}^3=\text{Et}$ )	3.6:1	7.0:1
<b>3.3e</b> ( $\text{R}^3=\text{Me}(\text{CH}_2)_5$ )	2.0:1	5.5:1
<b>3.3f</b> ( $\text{R}^3=\text{Me}(\text{CH}_2)_9$ )	2.0:1	5.7:1

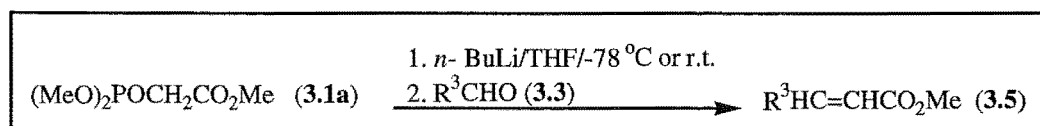
To our knowledge, this is the first report of such a pattern of variance for the reaction of phosphonates of the type **3.1** with unbranched aliphatic aldehydes (**3.3**) (see Scheme 3.13 for a general scheme). However, a literature investigation<sup>3,11</sup> of the reaction between the alpha-substituted phosphonate (**3.2**  $\text{R}, \text{R}^1=\text{Et}$ ,  $\text{R}^2=\text{Me}/\text{Et}$ ) and the aldehyde (**3.3**  $\text{R}^3=\text{Me}/\text{Et}$ ) showed that the proportion of *Z* product was significantly enhanced when  $\text{R}^3=\text{Et}$  rather than  $\text{R}^3=\text{Me}$ —i.e. for the longer chain aldehyde (see Scheme 3.13 for a general scheme). This was not particularly commented upon by the authors.

Scheme 3.13



The effect of the alkyl group of a non-conjugated aldehyde (**3.3**, where  $R^3$  is alpha-branched) on the stereochemical outcome of the HWE reaction was investigated, using the four aldehydes isobutyraldehyde (**3.3g**), 2-ethylbutyraldehyde (**3.3h**), cyclohexanecarbaldehyde (**3.3i**), and 2-methylundecanal (**3.3j**). As shown in Table 3.4, the aldehydes were reacted with the anion of trimethyl phosphonoacetate (**3.1a**), at  $-78\text{ }^{\circ}\text{C}$  and at r.t., in THF, using *n*-BuLi to generate the anion. The isomer ratios, presented in Table 3.4, demonstrate that the reaction with the alpha-branched aldehydes (**3.3g-3.3j**) gave a significant proportion of the *Z* product, both at  $-78\text{ }^{\circ}\text{C}$  and at r.t. This was most obvious at  $-78\text{ }^{\circ}\text{C}$ , since all the alpha-branched aldehydes studied (**3.3g-3.3j**, Table 3.4) gave a predominance of the *Z* product. Further, at r.t. a higher proportion of *Z* product was obtained with the alpha-branched aldehydes (**3.3g-3.3j**) than for unbranched aldehydes with a carbon chain of the same length. For example, the reaction of **3.1a** with the alpha-branched aldehyde (**3.3g**) resulted in an *E:Z* product ratio of 4.5:1 (Table 3.4), whereas reaction with the unbranched aldehyde (**3.3d**) gave a *E:Z* product ratio of 7.0:1 (Table 3.3).

Table 3.4. Product ratios obtained with Alpha-Branched Aliphatic Aldehydes (**3.3**)

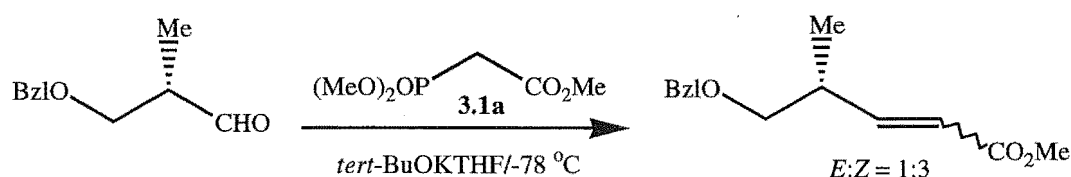


Aldehyde ( <b>3.3</b> )	<i>E:Z</i> Product Ratio	
	at $-78\text{ }^{\circ}\text{C}$	at r.t.
<b>3.3g</b> ( $R^3=(\text{Me})_2\text{CH}$ )	1:2.8	4.5:1
<b>3.3j</b> ( $R^3=\text{Me}(\text{CH}_2)_8(\text{Me})\text{CH}$ )	1:2.8	3.8:1
<b>3.3i</b> ( $R^3=\text{cyclohexyl}$ )	1:3.0	3.4:1
<b>3.3h</b> ( $R^3=(\text{Et})_2\text{CH}$ )	1:5.0	3.6:1



Interestingly, at  $-78\text{ }^{\circ}\text{C}$ , reaction of the anion of **3.1a** with isobutyraldehyde (**3.3g**) or with 2-methylundecanal (**3.3j**), resulted in identical *E:Z* product ratios of 1:2.8 (see Table 3.4). Both **3.3g** and **3.3j** have a methyl group at the 2-position. Scheme 3.16 details a report<sup>3,13</sup> of another aldehyde with a methyl group at the 2-position, reported to give an almost identical *E:Z* product ratio (on reaction with **3.1a**, under similar reaction conditions to the current study). However, when **3.1a** was reacted with 2-ethylbutyraldehyde (**3.3h**), which has an ethyl group at the 2-position, the *E:Z* isomer ratio decreased to 1:5.0 (Table 3.4). These observations suggest that, when the reaction is performed at  $-78\text{ }^{\circ}\text{C}$ , the important factor in determining the isomer ratio of the product may be the group at the 2-position of the aldehyde.

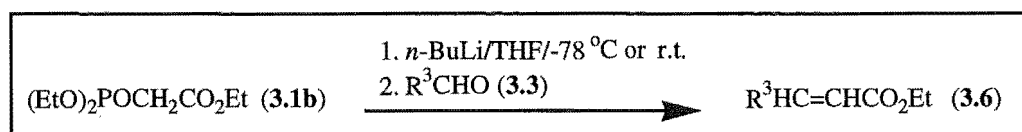
Scheme 3.16



To investigate, further, the influence of the aldehyde (**3.3**), the unbranched aldehydes (**3.3d** and **3.3e**), and the alpha-branched aldehydes (**3.3g** and **3.3h**), were reacted with the anion of triethyl phosphonoacetate (**3.1b**). The reactions were performed both at  $-78\text{ }^{\circ}\text{C}$  and at r.t., in THF, using *n*-BuLi to generate the anion (see Table 3.5). The data presented in Table 3.5 shows that both alpha-branched aldehydes (**3.3g** and **3.3h**) reacted with **3.1b** to produce a significant proportion of the *Z* product. This was also observed for the reaction of both **3.3g** and **3.3h** with the phosphonate (**3.1a**) (Table 3.4). The results for the reaction of the two unbranched aldehydes (**3.3d** and **3.3e**) with **3.1b** are also shown in Table 3.5. The reaction appeared to be relatively insensitive to the chain

length of the aldehyde. This was not observed for the reactions of **3.3d** and **3.3e** with the phosphonate (**3.1a**) (Table 3.3).

**Table 3.5. Product ratios obtained with the Phosphonate (3.1b)**



Aldehyde ( <b>3.3</b> )	E:Z Product Ratio	
	at -78 °C	at r.t.
<b>3.3d</b> (R <sup>3</sup> =Et)	9.3:1	19:1
<b>3.3e</b> (R <sup>3</sup> =Me(CH <sub>2</sub> ) <sub>5</sub> )	9.4:1	>19:1
<b>3.3g</b> (R <sup>3</sup> =(Me) <sub>2</sub> CH)	1.5:1	13:1
<b>3.3h</b> (R <sup>3</sup> =(Et) <sub>2</sub> CH)	1.5:1	12:1

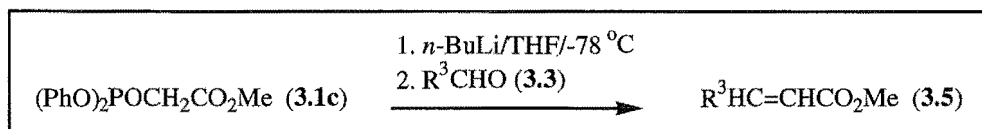
Further work in this area involved the investigation of methyl diphenylphosphonoacetate (**3.1c**). As shown in Table 3.6, the unbranched aldehydes (**3.3d** and **3.3e**) and the alpha-branched aldehydes (**3.3g**, **3.3h**, and **3.3j**) were reacted with the anion of **3.1c**. The reactions were performed both at -78 °C and at r.t., in THF, using *n*-BuLi to generate the anion. The results, presented in Table 3.6, demonstrate that for the unbranched aldehydes (**3.3d** and **3.3e**), the longer chain aldehyde (**3.3e**) reacted with **3.1c** to form a higher proportion of Z product. This was also observed when the aldehydes (**3.3d** and **3.3e**) were each reacted with the phosphonate (**3.1a**) (Table 3.3) but not with the phosphonate (**3.1b**) (Table 3.5).

The alpha-branched aldehydes (**3.3g**, **3.3h**, and **3.3j**) reacted with the phosphonate (**3.1c**) to form a higher proportion of the Z product than unbranched aldehydes of the same chain length (compare the results obtained with **3.3d** and **3.3g**, shown in Table 3.6). This

phenomenon was also observed for the reactions with **3.1a** (Tables 3.3 and 3.4) and with **3.1b** (Table 3.5).

As a consequence of our work on HIV protease inhibitors (Chapter 2), the aldehydes (**2.25a** and **2.25b**—Table 3.6) were both reacted with the anion of the phosphonate (**3.1c**). The reactions were performed at -78 °C and at r.t., in THF, using *n*-BuLi to generate the anion. The results are displayed in Table 3.6. The stereochemical outcome of the reaction of **2.25a** with the phosphonate (**3.1c**) was very similar to that obtained for heptaldehyde (**3.3e**) under the same conditions. Unexpectedly, the reaction of **3.1c** with **2.25b** resulted in a considerably smaller proportion of the *Z* isomer than that obtained for **2.25a**.

Table 3.6. Product ratios obtained with the Phosphonate (**3.1c**)

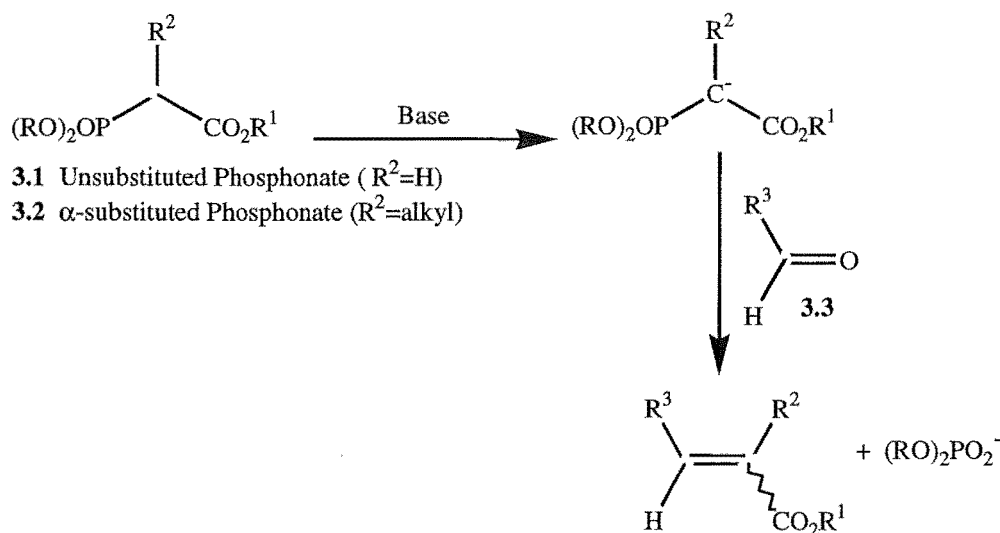


Aldehyde ( <b>3.3</b> )	<i>E</i> : <i>Z</i> Product Ratio
<b>3.3d</b> ( $\text{R}^3=\text{Et}$ )	1:1.3
<b>3.3e</b> ( $\text{R}^3=\text{Me}(\text{CH}_2)_5$ )	1:7.0
<b>3.3g</b> ( $\text{R}^3=(\text{Me})_2\text{CH}$ )	1:3.3
<b>3.3h</b> ( $\text{R}^3=(\text{Et})_2\text{CH}$ )	1:3.8
<b>3.3j</b> ( $\text{R}^3=\text{Me}(\text{CH}_2)_8(\text{Me})\text{CH}$ )	1:5.7
<b>2.25a</b> ( $\text{R}^3=\text{PhCH}_2\text{CH}_2$ )	1:6.4
<b>2.25b</b> ( $\text{R}^3=4\text{-nitroC}_6\text{H}_4\text{OCH}_2$ )	1:3.4

Overall, for the three phosphonates investigated (**3.1a**, **3.1b**, and **3.1c**), a high proportion of the *Z* product was obtained on reaction with alpha-branched aldehydes such as isobutyraldehyde (**3.3**  $\text{R}^3=(\text{Me})_2\text{CH}$ ). This is, to our knowledge, the first comprehensive study of phosphonates of the type **3.1** (Scheme 3.13) with alpha-branched aldehydes.

However, several investigators<sup>3,11, 3.12</sup> have reported significant increases in the proportion of Z product when alpha-branched aldehydes were reacted with alpha-substituted phosphonates of the type **3.2** (Scheme 3.13). For example, Seyden-Penne *et al.*<sup>3,12</sup> reported the reaction of alpha-branched aldehydes, such as isobutyraldehyde (**3.3**  $R^3=(Me)_2CH$ ), with the phosphonate (**3.2**  $R=Et$ ,  $R^1, R^2=Me$ ) to give stereoselectively Z olefins (see Scheme 3.13 for a general scheme). The reactions were performed with *n*-BuLi in THF at low temperature. Further, Kinstle *et al.*<sup>3.11</sup> showed that the reaction of the phosphonate (**3.2**  $R, R^1=Et$ ,  $R^2=Me/Et$ ) with the aldehyde (**3.3**  $R^3=Et/(Me)_2CH/tert-Bu$ ) gave a significant increase in the proportion of Z product when  $R^3=Et$  was replaced by  $R^3=(Me)_2CH$  (see Scheme 3.13 for a general scheme). The proportion decreased again when  $R^3=tert-Bu$ . These workers concluded that steric factors were responsible for the trends, the precise nature of which was not stated.<sup>3.11</sup>

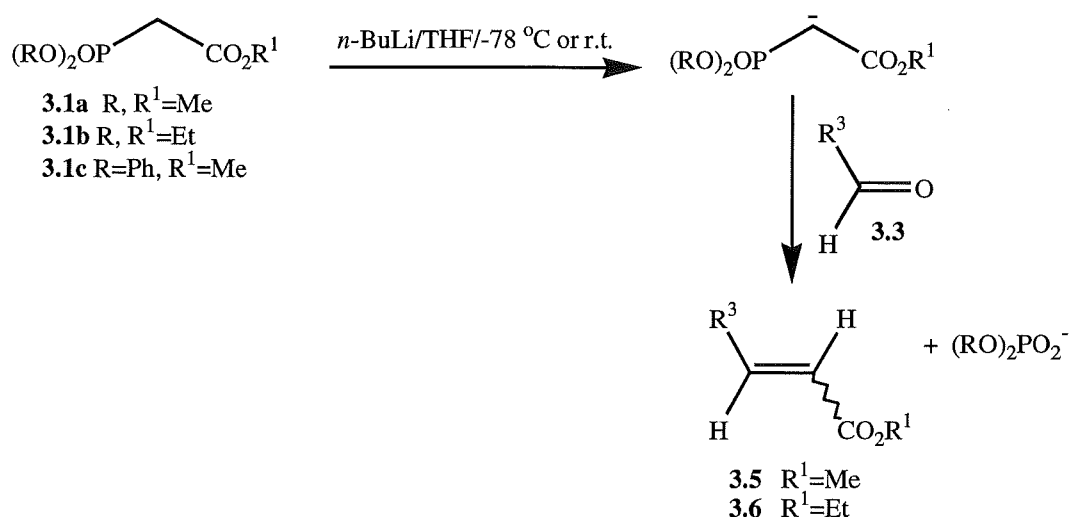
Scheme 3.13



Heathcock and Thompson<sup>3.7</sup> studied the reaction of unbranched and alpha-branched aldehydes with trimethyl phosphonoacetate (**3.1a**), under various conditions of solvent, temperature, and cation. These conditions included the use of *n*-BuLi in THF, at  $-78^\circ$  and at r.t., as was the case in our investigations (Scheme 3.14). Their results focused on the

finding that regardless of the reaction conditions, the aldehyde (**3.3**  $R^3 = \text{tert-Bu}$ ) resulted in the exclusive formation of the *E* olefin. This was attributed to a greater propensity for the formation of the intermediates giving rise to the *E* olefin (for steric or kinetic reasons,<sup>3,7</sup> the precise nature of which was not stated). However, their published data showed that, for every set of conditions studied, isobutyraldehyde (**3.3**  $R^3 = (\text{Me})_2\text{CH}$ ) gave a greater proportion of the *Z* olefin than the unbranched aldehyde (**3.3**  $R^3 = \text{CH}_2\text{CH}_2\text{C}(\text{Me})_2\text{CH}_2\text{CH}=\text{C}(\text{Me})_2$ ). This trend was not commented upon by the authors. Similarly, in the current study, a high proportion of the *Z* olefin was obtained for reactions of alpha-branched aldehydes such as isobutyraldehyde (**3.3g**) with the phosphonate (**3.1a**) and further with the phosphonates (**3.1b** and **3.1c**)—see Tables 3.4, 3.5, and 3.6.

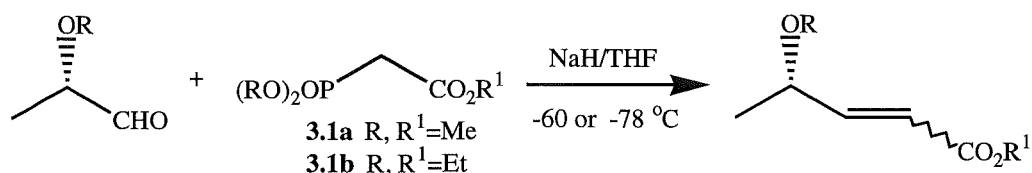
Scheme 3.14



## Part 2. Effect of the Phosphonate on the HWE Reaction

During our earlier investigations on the stereochemical outcome of the HWE reaction (Part 1) it was noted that, under identical conditions, trimethyl phosphonoacetate (**3.1a**) always produced a greater proportion of the *Z* olefin than triethyl phosphonoacetate (**3.1b**). This observation was supported by a literature report<sup>3.15</sup> where the phosphonates (**3.1a** and **3.1b**) were each reacted with an alpha-branched aldehyde as shown in Scheme 3.17. The reaction with **3.1a** resulted in a 1:1 ratio of isomeric olefins whereas reaction with **3.1b** gave an excess of the *E* olefin (12:1).

Scheme 3.17



To investigate this observation, studies were carried out using the four phosphonate reagents, trimethyl phosphonoacetate (**3.1a**), triethyl phosphonoacetate (**3.1b**), ethyl dimethylphosphonoacetate (**3.1d**), and methyl diethylphosphonoacetate (**3.1e**). These phosphonates were chosen to investigate the influence of the substituents (R) and (R<sup>1</sup>). The anions of these phosphonates (**3.1a**, **3.1b**, **3.1d**, and **3.1e**) were each reacted with heptaldehyde (**3.3e**), isobutyraldehyde (**3.3g**), and benzaldehyde (**3.3a**), as shown in Table 3.7. The reactions were performed in THF, at -78 °C, using *n*-BuLi to generate the anion (conditions previously established as conducive to the formation of the *Z* isomer as this was often the minor isomer).

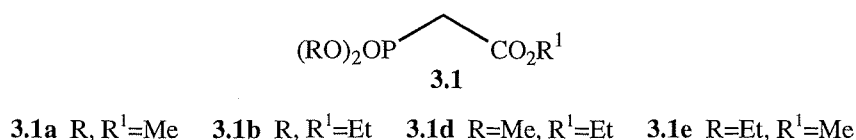
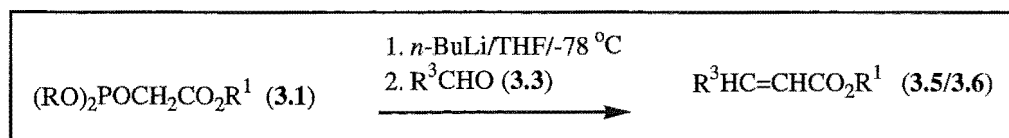


Table 3.7. Product Ratios obtained with the Phosphonates (**3.1a**, **3.1b**, **3.1d**, & **3.1e**)

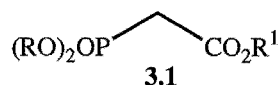
Aldehyde ( <b>3.3</b> )	Phosphonate Reagent ( <b>3.1</b> )		<i>E</i> / <i>Z</i> Product Ratio
		R      R <sup>1</sup>	
<b>3.3e</b> (R <sup>3</sup> =Me(CH <sub>2</sub> ) <sub>5</sub> )	<b>3.1a</b>	Me      Me	2.0:1
	<b>3.1d</b>	Me      Et	1.2:1
	<b>3.1b</b>	Et      Et	9.4:1
	<b>3.1e</b>	Et      Me	6.7:1
<b>3.3g</b> (R <sup>3</sup> =(Me) <sub>2</sub> CH)	<b>3.1a</b>	Me      Me	1:2.8
	<b>3.1d</b>	Me      Et	1:2.9
	<b>3.1b</b>	Et      Et	1.5:1
	<b>3.1e</b>	Et      Me	2.4:1
<b>3.3a</b> (R <sup>3</sup> =Ph)	<b>3.1a</b>	Me      Me	>19:1
	<b>3.1d</b>	Me      Et	>19:1
	<b>3.1b</b>	Et      Et	>19:1
	<b>3.1e</b>	Et      Me	>19:1

The results presented in Table 3.7 show that for each reaction, the stereochemical outcome was dependent on the nature of R. For example, reactions performed on isobutyraldehyde (**3.3g**) resulted in an almost threefold excess of the *Z* product with both **3.1a** and **3.1d** (where R is methyl), but with **3.1b** and **3.1e** (where R is ethyl) a significant excess of the *E* product resulted. However, the product ratio was relatively insensitive to whether the phosphonate had a methyl or an ethyl group at R<sup>1</sup>.

In summary, the results presented in Table 3.7, indicate that:

- (1) The group at R<sup>1</sup>, whether ethyl or methyl, had minimal effect on the stereochemical outcome of the reaction.

(2) The nature of R, whether ethyl or methyl, had an important influence on the stereochemical outcome of the reaction. In particular, R=methyl appeared to favour the formation of the Z isomer.



Literature reports<sup>3.16, 3.17</sup> substantiate our findings that, for the phosphonate (**3.1**), changes at R<sup>1</sup> had a minimal influence on the stereochemical outcome of HWE reactions. For example, Still<sup>3.16</sup> reported that HWE reactions are relatively insensitive to changes at R<sup>1</sup> of phosphonate reactant (**3.1**) and that only slightly elevated *E:Z* ratios were obtained when R<sup>1</sup> was cyclohexyl rather than methyl. Further, Wadsworth *et al.*<sup>3.17</sup> attempted to promote the formation of the Z isomer, by combining large naphthyl groups at R<sup>1</sup> and R<sup>3</sup> (Scheme 3.18). This strategy was designed to promote the formation of the *erythro* betaine (which gives rise to the Z isomer) for steric reasons (Figure 3.1), but was not particularly successful.<sup>3.17</sup>

Scheme 3.18

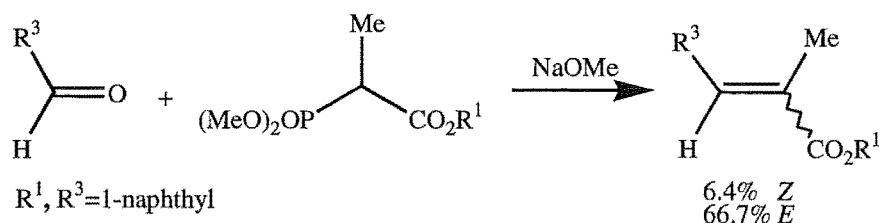
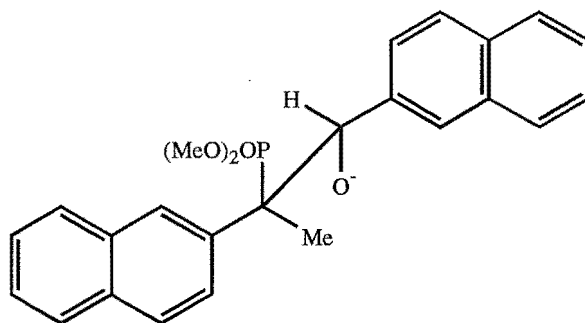
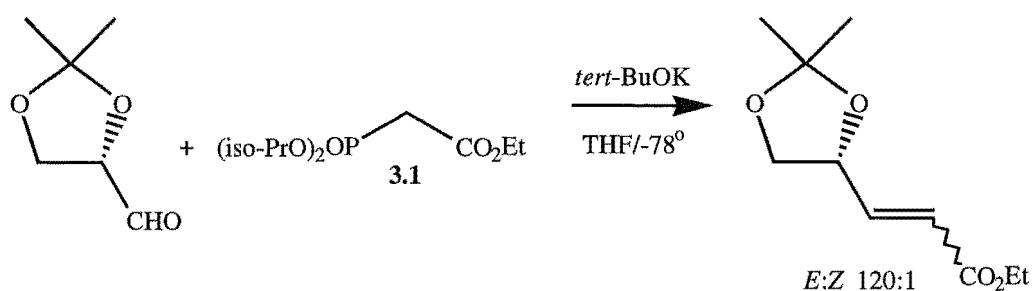




Figure 3.1. The use of naphthyl groups to promote the formation of the *erythro* betaine.

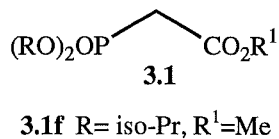
Literature reports<sup>3.13, 3.18</sup> support our finding that the R group of the phosphonate (**3.1**) had an important influence on the stereochemical outcome of the HWE reaction. Kishi *et al.* state that, in general, a phosphonate reagent with a large phosphonate ester group yields a predominance of the *E* olefin.<sup>3.13</sup> For example, the reaction of the phosphonate **3.1** (R=iso-Pr, R<sup>1</sup>=Et) with an alpha-branched aldehyde was reported to give a 120 times excess of the *E* product (Scheme 3.19).<sup>3.18</sup> The promotion of the *E* product by a large phosphoryl ester is consistent with our findings, shown in Table 3.7.

Scheme 3.19



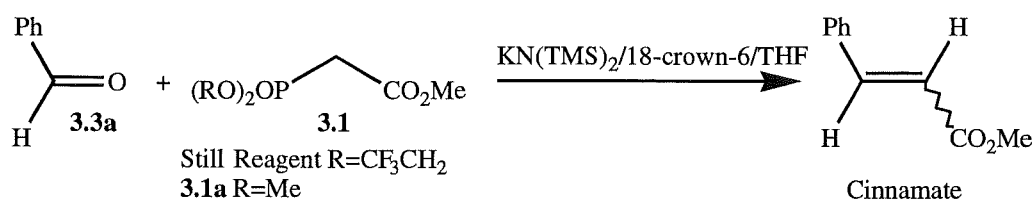
To corroborate these findings, the phosphonate (**3.1f**) was synthesised.<sup>3.19</sup> The anion of **3.1f** was reacted with each heptaldehyde (**3.3e**), isobutyraldehyde (**3.3g**), and benzaldehyde (**3.3a**). The reactions were again performed in THF, at -78 °C, using *n*-BuLi to generate the anion. The results, shown in Table 3.8, demonstrate that for each aldehyde (**3.3a**, **3.3e**, or **3.3g**), the reaction with **3.1f** led to a high proportion of *E*

product. In fact, Table 3.8 clearly shows that for the phosphonate (**3.1**), increasing the size of R, from Me (as in **3.1a**) to Et (as in **3.1e**) and to iso-Pr (as in **3.1f**), increased the proportion of *E* product.



These findings imply that the influence of the phosphonate ester is entirely steric. Examination of the literature,<sup>3,16</sup> however, reveals that this is only part of the story. For example, Still developed a phosphonate reagent (**3.1** R=F<sub>3</sub>CCH<sub>2</sub>), which yielded a dramatically higher proportion of the *Z* olefin (compared to the phosphonate (**3.1a**) under identical conditions) (Scheme 3.20).<sup>3,16</sup> For example, benzaldehyde (**3.3a**) was converted almost stereoselectively to *Z*-cinnamate by the Still reagent (**3.1** R=F<sub>3</sub>CCH<sub>2</sub>).<sup>3,16</sup> However with the phosphonate (**3.1a** R=Me), a 12 fold excess of *E*-cinnamate was produced (Scheme 3.20).<sup>3,16</sup> Clearly, steric factors alone do not account for these results.

Scheme 3.20



Further, a recent paper<sup>3,19</sup> reported a new phosphonate, ethyl diphenylphosphonoacetate (**3.1** R=Ph, R<sup>1</sup>=Et) which gave *Z* olefins with up to 90% selectivity. We suggested that the phenyl group of the phosphoryl ester may be responsible for the high *Z* selectivity. To demonstrate this, methyl diphenylphosphonoacetate (**3.1c**, R=Ph, R<sup>1</sup>=Me) was synthesised.<sup>3,19</sup> The reaction of the anion of **3.1c** with each heptaldehyde (**3.3e**),

isobutyraldehyde (**3.3g**), and benzaldehyde (**3.3a**) was studied and the results are presented in Table 3.8. These reactions were again performed in THF, at  $-78^{\circ}\text{C}$ , using *n*-BuLi to generate the anion. The results were compared to those obtained with trimethyl phosphonoacetate(**3.1a**), and with triethyl phosphonoacetate (**3.1b**), and with the literature result for the reaction of ethyl diphenylphosphonoacetate (**3.1**  $\text{R}=\text{Ph}$ ,  $\text{R}^1=\text{Et}$ ) with **3.3a** (Table 3.8).<sup>3,19</sup>

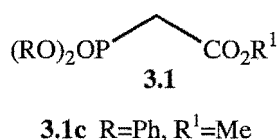
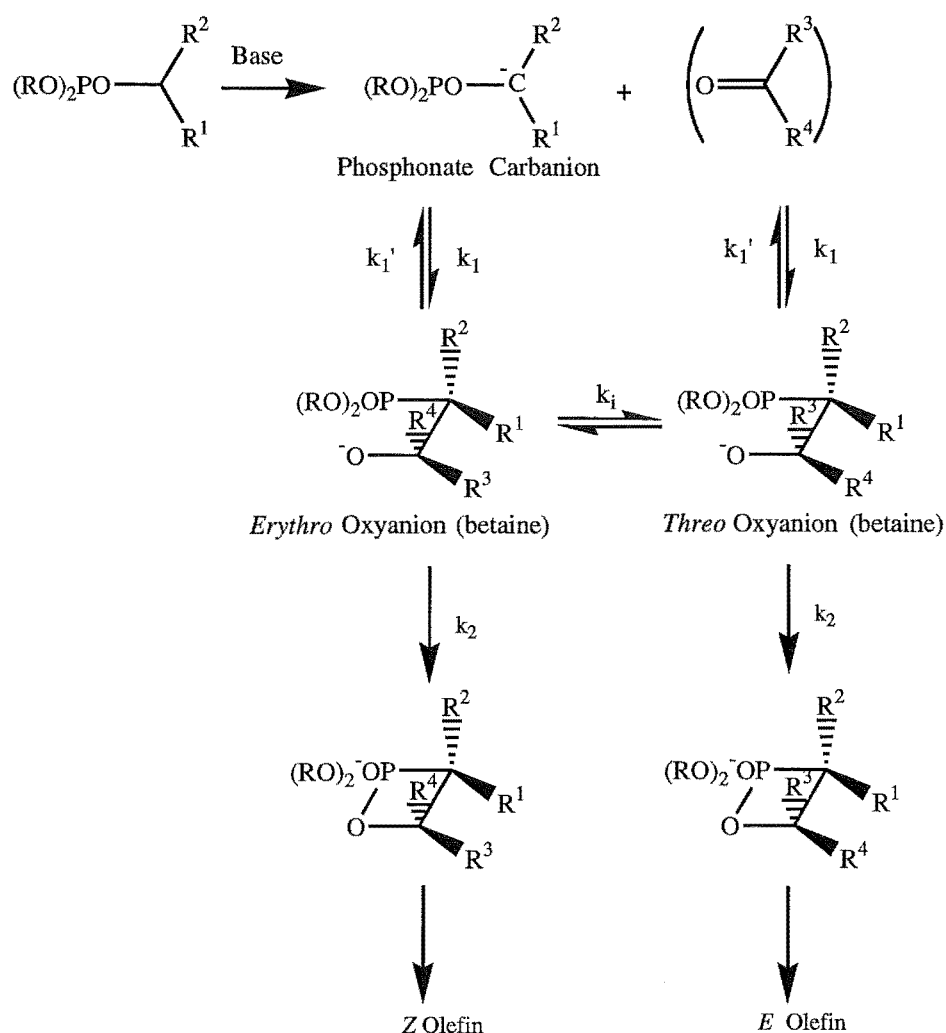


Table 3.8. Product ratios obtained with the Phosphonates (**3.1a**, **3.1c**, **3.1e**, & **3.1f**)

<div style="border: 1px solid black; padding: 10px; text-align: center;"> <math>(\text{RO})_2\text{POCH}_2\text{CO}_2\text{R}^1</math> (<b>3.1</b>)           <math>\xrightarrow[2. \text{R}^3\text{CHO (3.3)}]{1. n\text{-BuLi/THF/-78}^{\circ}\text{C}}</math> <math>\text{R}^3\text{HC=CHCO}_2\text{R}^1</math> (<b>3.5/3.6</b>)         </div>				
Aldehyde ( <b>3.3</b> )	Phosphonate ( <b>3.1</b> )	R	$\text{R}^1$	<i>E</i> : <i>Z</i> Product Ratio
<b>3.3e</b> ( $\text{R}^3=\text{Me}(\text{CH}_2)_5$ )	<b>3.1a</b>	Me	Me	2.0:1
	<b>3.1e</b>	Et		6.7:1
	<b>3.1f</b>	iso-Pr		>19:1
	<b>3.1c</b>	Ph		1:7.0
<b>3.3g</b> ( $\text{R}^3=(\text{Me})_2\text{CH}$ )	<b>3.1a</b>	Me	Me	1:2.8
	<b>3.1e</b>	Et		2.4:1
	<b>3.1f</b>	iso-Pr		10:1
	<b>3.1c</b>	Ph		1:3.3
<b>3.3a</b> ( $\text{R}^3=\text{Ph}$ )	<b>3.1a</b>	Me	Me	>19:1
	<b>3.1e</b>	Et		>19:1
	<b>3.1f</b>	iso-Pr		>19:1
	<b>3.1c</b>	Ph		1:2.2
<b>3.3a</b> ( $\text{R}^3=\text{Ph}$ )	<b>3.1d</b>	Me	Et	>19:1
	<b>3.1b</b>	Et		>19:1
	<b>3.1</b>	Ph		1:2.0 <sup>3,19</sup>

An examination of Table 3.8 shows that for phosphoryl esters, where R is aliphatic, a larger R group promoted the formation of the *E* product. However, when R was phenyl high *Z* selectivity resulted. For example, the reaction of isobutyraldehyde (**3.3g**) with **3.1f** (R=iso-Pr) gave a 10 fold excess of the *E* olefin, whereas with **3.1c** (R=Ph) a three fold excess of the *Z* olefin resulted. Such results could not be explained by steric considerations alone. Therefore, the electronic effect of the phenyl ring was considered.

Scheme 3.3

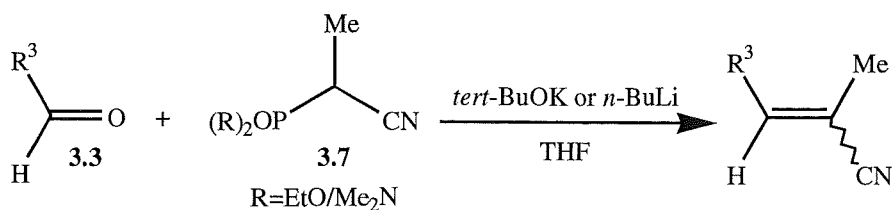


We hypothesised that the large increase in the amount of *Z* olefin produced, when the R group of the phosphoryl ester was phenyl, was due to an increase in the electrophilicity of

the phosphorous atom in the betaine intermediate (see Scheme 3.3). Since the lone pairs on the phenoxy oxygen are conjugated to the ring, less electron density is available to the phosphorous atom. Increased phosphorous atom electrophilicity in turn increases the rate of nucleophilic attack at the phosphorous. As already discussed in the introduction to this chapter, an increase in the rate of decomposition ( $k_2$ ) of the betaines to product, results in a decrease in the “reversibility factor” and this leads to an increase in the proportion of the *Z* product.

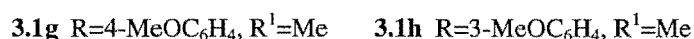
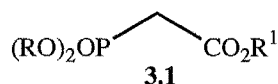
This explanation is supported by previous work by Deschamps *et al.*<sup>3,9</sup> where the phosphorous atom electrophilicity of various phosphonates was modified (by modifying the R group) resulting in changes to the *E:Z* product ratio. For example, the reaction of a number of aldehydes, such as benzaldehyde (**3.3a**), with the phosphonate (**3.7**) showed that when R was Me<sub>2</sub>N a greater proportion of the *E* olefin was produced than when R was OEt (Scheme 3.21).<sup>3,9</sup> Since a dimethylamino group is a better electron donor than an ethoxy group, the authors suggested that the phosphorous atom would be less electrophilic when R=Me<sub>2</sub>N.<sup>3,9</sup> This would make ring closure less attractive, reducing the rate of decomposition ( $k_2$ , Scheme 3.3) of the betaine intermediates to product.<sup>3,9</sup> Thus, there would be an increase in the “reversibility factor” and subsequently in the proportion of *E* product.<sup>3,9</sup>

Scheme 3.21



To investigate the importance of the electrophilicity of the phosphorous atom, two new phosphonates, (**3.1g** and **3.1h**), were synthesised. It was reasoned that:

- (1) for the phosphonate (**3.1g**), the presence of an electron donating substituent at the *para* position of the phenyl ring would decrease delocalisation of the phenoxy oxygen lone pair into the ring and hence make the phosphorous less electrophilic. Thus on reaction with an aldehyde, such as **3.3g**, an increase in proportion of the *E* product may be obtained (as compared to **3.1c**, where the phenyl ring is unsubstituted).
- (2) for the phosphonate (**3.1h**), an electron donating substituent at the *meta* position of the phenyl ring would not effect the delocalisation of electron density from the phenoxy oxygen and therefore a similar result (on reaction with an aldehyde such as **3.3g**) may be expected to that obtained for the phosphonate (**3.1c**).



The anions of the new phosphonates (**3.1g** and **3.1h**) were each reacted with heptaldehyde (**3.3e**), isobutyraldehyde (**3.3g**), and benzaldehyde (**3.3a**). The results are shown in Table 3.9. The reactions were again performed in THF, at -78 °C, using *n*-BuLi to generate the anion. The results obtained were exactly in agreement with our predictions. In particular, reaction with the phosphonate (**3.1g**), which has the electron donating substituent at the *para* position of the phenyl ring, resulted in an increase in the *E:Z* ratio (relative to **3.1c** where the phenyl ring is unsubstituted) for the aldehydes (**3.3a**, **3.3e**, and **3.3g**). For example, the reaction of **3.3e** with **3.1g** gave a four fold excess of the *Z* olefin, whereas with **3.1c**, a seven fold excess was observed. Further, the results for the reaction of the phosphonate (**3.1h**), where the electron donating substituent is at the *meta*

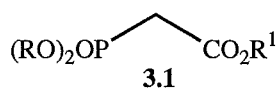
position of the phenyl ring, were very similar to the those obtained for the phosphonate (3.1c) (with each of 3.3a, 3.3e, and 3.3g). For example, reaction of 3.3g with either 3.1h or 3.1c both resulted in a *E:Z* product ratio of 1:3.3.

**Table 3.9. Product ratios obtained with the Phosphonates (3.1c, 3.1g & 3.1h)**

<div style="border: 1px solid black; padding: 10px; display: inline-block;"> <math display="block">(RO)_2POCH_2CO_2R^1 \text{ (3.1)} \xrightarrow[2. R^3CHO \text{ (3.3)}]{1. n-BuLi/THF/-78^\circ C} R^3HC=CHCO_2R^1 \text{ (3.5)}</math> </div>		
Aldehyde (3.3)	Phosphonate Reagent (3.1, R <sup>1</sup> =Me)	<i>E:Z</i> Isomer Ratio
<b>3.3e</b> (R <sup>3</sup> =Me(CH <sub>2</sub> ) <sub>5</sub> )	<b>3.1g</b> (R=4-MeOC <sub>6</sub> H <sub>4</sub> )	1:4.2
	<b>3.1h</b> (R=3-MeOC <sub>6</sub> H <sub>4</sub> )	1:7.2
	<b>3.1c</b> (R=Ph)	1:7.0
<b>3.3g</b> (R <sup>3</sup> =(Me) <sub>2</sub> CH)	<b>3.1g</b> (R=4-MeOC <sub>6</sub> H <sub>4</sub> )	1:2.1
	<b>3.1h</b> (R=3-MeOC <sub>6</sub> H <sub>4</sub> )	1:3.3
	<b>3.1c</b> (R=Ph)	1:3.3
<b>3.3a</b> (R <sup>3</sup> =Ph)	<b>3.1g</b> (R=4-MeOC <sub>6</sub> H <sub>4</sub> )	1:1.3
	<b>3.1h</b> (R=3-MeOC <sub>6</sub> H <sub>4</sub> )	1:2.4
	<b>3.1c</b> (R=Ph)	1:2.2

The appearance of a recent report substantiates this work.<sup>3.20</sup> Reddy *et al.* on studying the reaction of various phosphonates (3.1) with a variety of aldehydes, including benzaldehyde (3.3a), have shown that phosphonates, such as 3.1i and 3.1j, which have an electron withdrawing group attached to the phosphorous atom, lead to *Z* selective olefination (at -78 °C).<sup>3.20</sup> For example, reaction of the anion of either 3.1i or 3.1j with benzaldehyde (3.3a) resulted in an 95% excess of the *Z* olefin.<sup>3.20</sup> These reactions were performed in *N,N*-dimethylformamide or THF, at -78 °C and the anion was generated using either sodium hydride or potassium *tertiary*-butoxide. The authors' rationale for this observed *Z* selectivity is that "the equilibration of the initial adduct formed with [the]

carbanion and [the] aldehyde is slowed down and the subsequent step of cyclization and elimination is accelerated".<sup>3.20</sup>



**3.1i** R=4-nitroC<sub>4</sub>H<sub>6</sub>, R<sup>1</sup>=Me      **3.1j.** R=Cl<sub>3</sub>CH<sub>2</sub>C, R<sup>1</sup>=Me

Overall, we concluded that for the phosphonates (**3.1c**, **3.1g**, and **3.1h**), where R is an aromatic group, electronic factors were essentially responsible for the high *Z* selectivity obtained on reaction with various aldehydes such as isobutyraldehyde (**3.3g**). However, for phosphonates where R is an aliphatic group (**3.1a**, **3.1b**, **3.1d**, **3.1e**, and **3.1f**), the stereochemical outcome of the reaction appeared to be controlled by steric considerations, such that a larger R group resulted in an increase in the proportion of *E* product.



## Part 3. Effect of Temperature on the HWE Reaction

For every reaction presented here, regardless of the phosphonate (**3.1**) or the aldehyde (**3.3**), the proportion of *Z* product was greater for reactions carried out at -78 °C (rather than at r.t.). For example, as shown in Table 3.4 (part 1), the reaction of isobutyraldehyde (**3.3g**) with the phosphonate (**3.1a**) (in THF, with *n*-BuLi as the base) resulted in a three fold excess of the *Z* olefin at -78 °C. However, a four and a half fold excess of the *E* olefin was obtained when the reaction was performed at r.t. Heathcock and Thompson<sup>3.7</sup> report almost identical stereochemical results for these reactions. They attributed the increase in the *Z* olefin, at low temperature, to a decrease in reversibility of betaine formation, giving ratios which resemble the kinetic ratios rather than the thermodynamic ratios resembled at r.t.<sup>3.7</sup>

Table 3.10. The *E*:*Z* product ratio verses Temperature

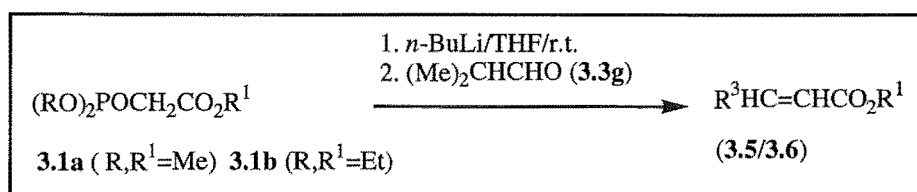
<div style="border: 1px solid black; padding: 10px; display: inline-block;"> <math display="block">(\text{MeO})_2\text{POCH}_2\text{CO}_2\text{Me} \text{ (3.1a)} \xrightarrow[2. (\text{Et})_2\text{CHCHO (3.3h)}]{1. n\text{-BuLi/THF}} (\text{Et})_2\text{CHHC=CHCO}_2\text{Me (3.5)}</math> </div>		
Temperature (°C)	<i>E</i> : <i>Z</i> Product Ratio	
25	3.6:1	
0	2:1	
-23	1:2.8	
-46	1:3.0	
-78	1:5	

Table 3.10 shows the variance in the *E*:*Z* product ratio over a range of temperatures for the reaction of 2-ethylbutyraldehyde (**3.3h**) with the phosphonate (**3.1a**). The reactions were performed with *n*-BuLi, in THF. Overall, the proportion of *Z* olefin decreased when the reaction temperature was increased, however, a similar stereochemical result was obtained when the reaction was performed at either -23 °C or at -46 °C.

## Part 4. Effect of the Solvent on the HWE Reaction

To investigate the influence of the solvent on the stereochemical outcome of the HWE reaction, isobutyraldehyde (**3.3g**) was reacted with the anion of the phosphonates (**3.1a** and **3.1b**). The reactions were performed at r.t., with *n*-BuLi, in either THF or benzene. The results, displayed in Table 3.11, demonstrate that the use of benzene (rather than THF) led to a significant increase in the amount of *E* product. For example, in the reaction of isobutyraldehyde (**3.3g**) with the phosphonate (**3.1a**), the *E*:*Z* isomer ratio increased from 4.5:1 to 10:1 when the solvent was changed from THF to benzene. Heathcock and Thompson also observed a sharp increase in *Z* olefin formation for reactions in THF in comparison to reactions in 1,2-dimethoxyethane (DME), ether, or benzene.<sup>3.7</sup>

Table 3.11. Product ratios obtained with THF and Benzene



Phosphonate ( <b>3.1</b> )	Solvent	<i>E</i> : <i>Z</i> ratio
<b>3.1b</b> ( $\text{R}/\text{R}^1 = \text{Et}$ )	THF	13:1
<b>3.1b</b>	Benzene	>19:1
<b>3.1a</b> ( $\text{R}/\text{R}^1 = \text{Me}$ )	THF	4.5:1
<b>3.1a</b>	Benzene	10:1
<b>3.1a</b>	DME	12:1 <sup>3.7</sup>

## Part 5. Experimental Conditions and Structure Assignment

The reaction between the phosphonate (**3.1**) and the aldehyde (**3.3**) was performed according to the general procedure described by Heathcock and Thompson.<sup>3,7</sup> In most examples the reaction was carried out in THF, either at r.t. or at  $-78\text{ }^{\circ}\text{C}$ , using *n*-BuLi to generate the anion of the phosphonate (**3.1**) (Scheme 3.14). A small excess of the phosphonate (**3.1**) was used (1.2 equivalents, typically 250 mg), to ensure that all the aldehyde (**3.3**) was reacted, as it was observed that some of the aldehydes (**3.3**) were not readily separable from the reaction products (**3.5** or **3.6**) by chromatography. The *E*:*Z* isomer ratio of the olefin products (**3.5** or **3.6**) was determined by measurement of the integrals of the olefin protons in the  $^1\text{H}$  NMR spectra of the crude reaction mixtures. When the reaction was repeated under identical conditions the stereochemical outcome was found to be reproducible.

Scheme 3.14

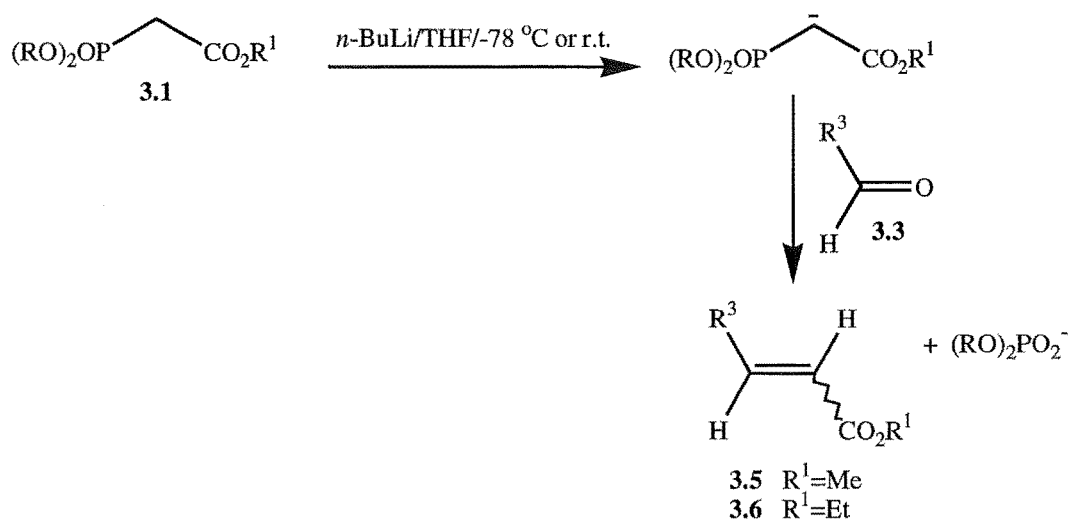


Table 3.12 Numbering of the Olefins (3.5/3.6, Scheme 3.14)

Olefin ( $R^3CH=CHCO_2R^1$ )	$R^1$	$R^3$
<b>3.5a</b>	Me	Ph
<b>3.6a</b>	Et	Ph
<b>3.5b</b>	Me	PhCH=CH
<b>3.6b</b>	Et	PhCH=CH
<b>3.5c</b>	Me	Me
<b>3.5d</b>	Me	Et
<b>3.6d</b>	Et	Et
<b>3.5e</b>	Me	Me(CH <sub>2</sub> ) <sub>5</sub>
<b>3.6e</b>	Et	Me(CH <sub>2</sub> ) <sub>5</sub>
<b>3.5f</b>	Me	Me(CH <sub>2</sub> ) <sub>9</sub>
<b>3.5g</b>	Me	(Me) <sub>2</sub> CH
<b>3.6g</b>	Et	(Me) <sub>2</sub> CH
<b>3.5h</b>	Me	(Et) <sub>2</sub> CH
<b>3.6h</b>	Et	(Et) <sub>2</sub> CH
<b>3.5i</b>	Me	cyclohexyl
<b>3.5j</b>	Me	Me(CH <sub>2</sub> ) <sub>8</sub> (Me)CH

The olefin products (**3.5a**, **3.5b**, **3.5e**, **3.5f**, **3.5h-i**, **3.6a**, **3.6b**, **3.6e**, and **3.6h**) were isolated by radial chromatography in order to determine the reaction yield. In the case of **3.5j**, the *E* and *Z* olefins were obtained separately. The lower molecular weight products (**3.5c**, **3.5d**, **3.5g**, **3.6d**, and **3.6g**) proved to be volatile and the yield tended to reflect the amount of time spent on the rotary evaporator. Since the main purpose of the work was to determine the stereochemical ratio, not the yield, isolation of these volatile products was not generally attempted.

The olefin products (**3.5** or **3.6**) were either identified by comparison of their spectra to published data (see Table 3.13) or where literature data was not obtainable (for **3.5e** (*Z*), **3.5f**, **3.5h**, **3.5i**, **3.5j**, and **3.6e** (*Z*)), the compounds were fully characterised.

Table 3.13. References to published data for the olefins (3.5/3.6)

Compound	Isomer	Reference
<b>3.5a</b>	<i>E</i>	FTIR data <sup>3.21</sup>
	<i>Z</i>	<sup>1</sup> H NMR data <sup>3.22</sup>
<b>3.6a</b>	<i>E</i>	<sup>1</sup> H NMR data <sup>3.23</sup>
<b>3.5b</b>	<i>E,E</i>	<sup>13</sup> C NMR data <sup>3.24</sup>
<b>3.5c</b>	<i>E</i>	<sup>1</sup> H NMR data <sup>3.25</sup>
	<i>Z</i>	<sup>1</sup> H NMR data <sup>3.26</sup>
<b>3.5d</b>	<i>E</i>	<sup>1</sup> H NMR data <sup>3.27</sup>
	<i>Z</i>	<sup>1</sup> H NMR data <sup>3.27</sup>
<b>3.6d</b>	<i>E</i>	<sup>1</sup> H NMR data <sup>3.28</sup>
	<i>Z</i>	<sup>1</sup> H NMR data <sup>3.28</sup>
<b>3.5e</b>	<i>E</i>	<sup>1</sup> H NMR data <sup>3.29</sup>
<b>3.6e</b>	<i>E</i>	<sup>1</sup> H NMR data <sup>3.30</sup>
<b>3.5g</b>	<i>E</i>	<sup>1</sup> H NMR data <sup>3.31</sup>
	<i>Z</i>	<sup>1</sup> H NMR data <sup>3.31</sup>
<b>3.6g</b>	<i>E</i>	<sup>1</sup> H NMR data <sup>3.28</sup>
	<i>Z</i>	<sup>1</sup> H NMR data <sup>3.28</sup>
<b>3.6h</b>	<i>E</i>	<sup>1</sup> H NMR data <sup>3.32</sup>
	<i>Z</i>	<sup>1</sup> H NMR data <sup>3.32</sup>

An examination of the <sup>1</sup>H NMR spectra of the olefins, for which published data was not available (**3.5e** (*Z*), **3.5f**, **3.5h**, **3.5i**, **3.5j**, and **3.6e** (*Z*)), enabled the assignment of the *E* and *Z* isomers. The assignment of *E* and *Z* olefins, on the basis of the coupling constants of the olefin protons in <sup>1</sup>H NMR spectra, is well documented.<sup>3.33</sup> To illustrate this, the <sup>1</sup>H NMR signals of the olefin protons of **3.5** are presented in Table 3.14. All signals assigned to the olefin protons of the *E* isomer had coupling constants between 15.1 and 17.4 Hz and all signals assigned to the olefin protons of the *Z* isomer had coupling constants between 10.2 and 11.8 Hz. These values are consistent with literature data for the coupling constants of *E* and *Z* olefinic protons.<sup>3.33</sup> Further, the position of the signals for HA and HC was diagnostic of the stereochemistry of the olefins (**3.5** or **3.6**) (see Figure 3.2 for definition of HA, HB, and HC). To illustrate this, the chemical shifts of the signals for HA, HB, and HC are presented in Table 3.14. For each olefin (**3.5**), the signal for HA was shifted downfield significantly (between 0.6 and 0.9 ppm) for the

*E* isomer relative to the *Z* isomer. This shift was attributed to the deshielding effect of the carbonyl group.<sup>3,34</sup> Further, the position of the signal for HC was shifted downfield significantly (between 0.3 and 1.3 ppm.) in the *Z* isomer relative to the *E* isomer, also attributed to deshielding by the carbonyl group.<sup>3,34</sup> Figure 3.2 shows that HA is close to the carbonyl group in the *E* isomer and not in the *Z* isomer, whereas HC is close to the carbonyl in the *Z* isomer.

**Table 3.14. <sup>1</sup>H NMR Signals (ppm) and for HA, HB, and HC (3.5)**

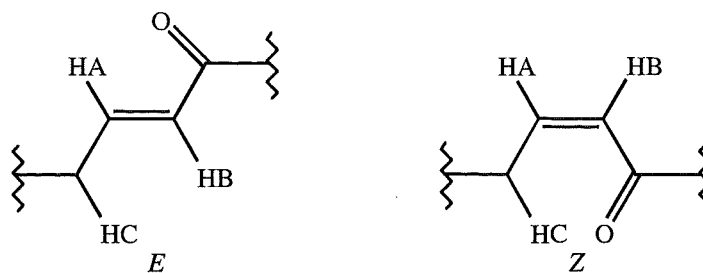
(Coupling Constants are in Hz)

Compound	<i>E</i> isomer			<i>Z</i> isomer		
	HA	HB	HC	HA	HB	HC
<b>3.5c</b>	6.95 (J=15.1)	5.81 (J=15.6)	1.81	6.31 (J=10.8)	5.77 #	2.10
<b>3.5d</b>	6.97 (J=15.6)	5.76 (J=15.6)	2.18	6.16 (J=11.7)	5.68 (J=11.2)	2.60
<b>3.5e</b>	6.96 (J=15.6)	5.81 (J=15.6)	2.18	6.23 *(J=11.8)	5.76 (J=11.3)	2.64
<b>3.5f</b>	6.93 (J=15.6)	5.77 (J=15.6)	2.14	6.18 (J=11.2)	5.72 (J=11.8)	2.61
<b>3.5g</b>	6.94 (J=16.2)	5.76 (J=16.1)	2.43	6.00 (J=11.7)	5.64 (J=11.2)	3.58
<b>3.5h</b>	6.73 (J=15.6)	5.78 (J=15.6)	1.94	5.89 *(J=11.7)	5.81 (J=11.7)	3.26
<b>3.5i</b>	6.92 (J=17.3)	5.76 (J=17.4)	2.10	6.03 (J=10.2)	5.66 (J=10.7)	3.29
<b>3.5j</b>	6.86 (J=15.2)	5.77 (J=15.6)	2.27	5.97 (J=11.3)	5.70 (J=10.8)	3.45

\*Coupling constant calculated after irradiation at HC

# Coupling constant not able to be calculated due to overlap of the signal for HB (*E* isomer).

Figure 3.2. Identification of HA, HB, and HC



Allylic coupling was observed, in the signals for HB, in the  $^1\text{H}$  NMR spectra of **3.5j** (*E*), **3.5e** (*Z*), and **3.5j** (*Z*). The coupling constants were as follows:

1.4 Hz (**3.5j** (*E*))

1.5 Hz (**3.5e** (*Z*))

0.9 Hz (**3.5j** (*Z*))

For each compound where literature  $^1\text{H}$  NMR data was not available (**3.5e** (*Z*), **3.5f**, **3.5h**, **3.5i**, **3.5j**, and **3.6e** (*Z*)—see Table 3.12 for numbering), the signals for HA, HB, and HC were irradiated to ensure their correct assignment. The results are summarised in Table 3.15. For each compound, irradiation of the signal for HC collapsed the signal for HA (to a doublet), irradiation of the signal for HA collapsed the signal for HB and HC, and irradiation of the signal for HB collapsed the signal for HA.

Table 3.15. Decoupling experiments on HA, HB, and HC (3.5 and 3.6)

Compound	Signal Irradiated (ppm)	Signal Collapsed (ppm) (J in Hz.)
<b>3.5e (Z)</b>	6.23 (HA)	5.76 (HB) to d (J=1.5) and 2.64 (HC) to t (J=7.7)
	5.76 (HB)	6.23 (HA) to t (J=7.8)
	2.64 (HC)	6.23 (HA) to d (J=11.8)
<b>3.5f (E)</b>	6.93 (HA)	5.77 (HB) to s and 2.14 (HC) to m
	5.77 (HB)	6.93 (HA) to m
	2.14 (HC)	6.93 (HA) to d (J=15.6)
<b>3.5f (Z)</b>	6.18 (HA)	5.72 (HB) to s and 2.61 (HC) to t (J=7.3)
	5.72 (HB)	6.18 (HA) to m
	2.61 (HC)	6.18 (HA) to d (J=11.2)
<b>3.5h (E)</b>	6.73 (HA)	5.78 (HB) to s and 1.94 (HC) to m
	5.78 (HB)	6.73 (HA) to d (J=8.8)
	1.94 (HC)	6.73 (HA) to d (J=15.6)
<b>3.5h (Z)</b>	5.89 (HA)	3.26 (HC) to m
	3.26 (HC)	5.89 (HA) to d (J=11.7)
	*Irradiation of HB not performed due to overlap of HA and HB signals	
<b>3.5i (E)</b>	6.92 (HA)	5.76 (HB) to s and 2.10 (HC) to m
	5.76 (HB)	6.92 (HA) to d (J=6.8)
	2.10 (HC)	6.92 (HA) to d (J=17.3)
<b>3.5i (Z)</b>	6.03 (HA)	5.66 (HB) to s and 3.29 (HC) to m
	5.66 (HB)	6.03 (HA) to d (J=9.8)
	3.29 (HC)	6.03 (HA) to d (J=10.2)
<b>3.5j (E)</b>	6.87 (HA)	5.77 (HB) to d (J=1.4) and 2.27 (HC) to m
	5.77 (HB)	6.87 (HA) to d (J=7.8)
	2.27 (HC)	6.87 (HA) to d (J=15.2)
<b>3.5j (Z)</b>	5.97 (HA)	5.70 (HB) to d (J=0.9) and 3.45 (HC) to m
	5.70 (HB)	5.97 (HA) to d (J=10.2)
	3.45 (HC)	5.97 (HA) to d (J=11.3)
<b>3.6e (Z)</b>	6.21 (HA)	5.74 (HB) to s and 2.62 (HC) to t (J=7.8)
	5.74 (HB)	6.21 (HA) to t (J=7.8)
	2.62 (HC)	6.21 (HA) to d (J=11.3)

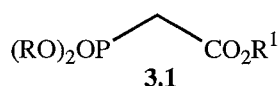


The  $^{13}\text{C}$  NMR spectra were consistent with the olefins (**3.5e** (*Z*), **3.5f**, **3.5h**, **3.5i**, **3.5j**, and **3.6e** (*Z*)—Table 3.12) and were assigned on the basis of chemical shift. The signals for the olefin carbons appeared between  $\delta$  156.3 and  $\delta$  149.2 ( $\text{COCH}=\underline{\text{CH}}$ ) and between  $\delta$  120.7 and  $\delta$  117.0 ( $\text{COCH}$ ). The chemical shift of the signal for the methyl ester (**3.5**) showed very little variation between the compounds: approximately 1 ppm for the CO carbon ( $\delta$  167.5–166.1) and less than 1 ppm for the Me carbon ( $\delta$  51.0–50.3.). A distortionless enhancement by polarisation transfer (DEPT) experiment was performed to distinguish the alkyl carbons of **3.5j** (*E*). This allowed the assignment of the CHMe carbon to the signal at  $\delta$  36.4, the  $\text{CH}\underline{\text{Me}}$  carbon to the signal at  $\delta$  19.4, and the  $\text{CH}_2$ Me carbon at  $\delta$  14.0 (the olefin carbons were used as the reference carbons). The eight signals between  $\delta$  35.9 and  $\delta$  22.5 were shown by the DEPT to be due to methylene groups and were assigned to the eight CH $_2$  carbons of the alkyl chain. The  $^{13}\text{C}$  NMR spectrum of **3.5j** (*Z*) was assigned by comparison to that of **3.5j** (*E*). In the  $^{13}\text{C}$  NMR spectrum of **3.5e** (*Z*), only three signals attributable to  $\text{CH}_2$  carbons were observed. However, the peak at  $\delta$  28.8 ppm was disproportionately large and likely due to more than one  $\text{CH}_2$  group.

High resolution mass spectra (HRMS), obtained for **3.5e**, **3.5f**, **3.5h**, **3.5i**, **3.5j**, and **3.6e**, were consistent with the calculated mass. Since the *E* and *Z* isomers of **3.5e**, **3.5f**, **3.5h**, **3.5i**, and **3.6e** were not readily separable by radial chromatography, HRMS was performed on isomeric mixtures. The *E:Z* mixtures of **3.5f**, **3.5h**, and **3.5i** were separable by gas chromatography mass spectrometry (GCMS) to give low resolution MS data on each separate isomer (the minor isomer, separated by GCMS, was assumed to be the minor isomer as shown by the  $^1\text{H}$  NMR spectrum of the mixture). Interestingly, the fragmentation patterns of each isomer were slightly different. For example, for both **3.5h**

(*E*) and **3.5i** (*E*), the ion  $M^+-(MeOH)$  was a significant contributor to the spectrum. This is presumably due to the availability of a proton in the *E* arrangement of the double bond. Whereas in the MS of **3.5h** (*Z*) and **3.5i** (*Z*),  $M^+-(Me)$  and  $M^+-(MeO)$  were both significant ions.

The phosphonate reagents were either purchased (if commercially available) or synthesised by the method of Ando.<sup>3.19</sup> For the synthesised phosphonates (**3.1c**, **3.1f**, **3.1g**, and **3.1h**), the isolation procedure was modified slightly to omit the washing of the filtered reaction mixture with NaOH and brine since it was observed that some of the phosphonates were soluble in aqueous media. The synthesis<sup>3.19</sup> of the phosphonate (**3.1**,  $R=tert\text{-}Bu$ ,  $R^1=Me$ ) was attempted but no desired product was formed. Perhaps this phosphonate does not form readily, due to steric crowding around the phosphorous by the bulky *tert*-butyl group.



**3.1c**  $R=Ph$ ,  $R^1=Me$    **3.1f**  $R=iso\text{-}Pr$ ,  $R^1=Me$    **3.1g**  $R=4\text{-}MeOC_6H_4$ ,  $R^1=Me$    **3.1h**.  $R=3\text{-}MeOC_6H_4$ ,  $R^1=Me$

A literature search revealed that the phosphonates **3.1g** and **3.1h** were previously unknown and they were, therefore, fully characterised. In the  $^1H$  NMR spectra of **3.1g** and **3.1h** the signals for the  $\underline{CH}_2$  protons appeared as doublets ( $J=21.5$  Hz) due to coupling to the phosphorous atom. The  $^{13}C$  NMR spectra also displayed phosphorous coupling. For **3.1g** and **3.1h**, the signals due to the  $\underline{CH}_2$  and  $CH\underline{CO}$  carbons appeared as doublets ( $J=136.7\text{-}140.8$  Hz ( $\underline{CH}_2$ ) and  $8.3$  Hz ( $CH\underline{CO}$ )). In fact, the  $CH\underline{CO}$  carbon was assigned on the basis of this coupling. For **3.1h** and **3.1g**, the signal for the  $\underline{CO}_2$  carbon appeared as a doublet ( $J=6.3$  Hz).

## Experimental

### General Procedure

Nuclear Magnetic Resonance (NMR) spectra were recorded on either a Varian CFT300 or a XT300 spectrometer. Mass spectra (MS) were obtained on a Kratos MS80RFA spectrometer. Infrared (IR) spectra were recorded on a Shimadzu FTIR-8201PC spectrophotometer. Radial chromatography was performed on a Chromatotron (Harrison and Harrison) using Merck type 60 PF254 silica gel. The aldehydes (**3.3a-j**) and the phosphonates (**3.1a**, **3.1b**, **3.1d**, and **3.1e**) were commercially available.

### General Procedure<sup>3.7</sup> for Reactions with Trimethyl phosphonoacetate (**3.1a**)

A stirring solution of 250 mg (1.4 mmol) of trimethyl phosphonoacetate (**3.1a**) in 2.5 ml of dry tetrahydrofuran (THF) was cooled to 0 °C under a nitrogen atmosphere and 0.88 ml (1.4 mmol) of a 1.6 M solution of *n*-butyllithium (*n*-BuLi) in hexane was added dropwise. After 10 minutes, the reaction was brought to the desired temperature and the aldehyde (1.2 mmol) was added dropwise. The resultant mixture was stirred for 1.5 hours, quenched by the addition of 3.0 ml of water, and further diluted with 5.0 ml of ether. The aqueous layer was removed and the organic layer was washed with saturated brine, dried with magnesium sulfate (MgSO<sub>4</sub>), filtered, and the solvent was evaporated under reduced pressure. The crude product was analysed directly by <sup>1</sup>H NMR and where indicated, purified by radial chromatography on silica (80% petroleum ether, 20% ethyl acetate).

Preparation of Methyl cinnamate (3.5a)

The reaction was performed as described by the general procedure, at -78 °C, with 127 mg (1.2 mmol) of benzaldehyde (**3.3a**). The *E:Z* ratio of the crude product (**3.5a**) was found to be >19:1, by <sup>1</sup>H NMR. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave **3.5a** (*E*) as a colourless oil (quantitative recovery). The FTIR spectrum of **3.5a** (*E*) was identical to published data.<sup>3,21</sup>

Preparation of Methyl 5-phenyl-2,4-pentadienoate (3.5b)

The reaction was performed as described by the general procedure, at -78 °C, with 158 mg (1.2 mmol) of cinnamaldehyde (**3.3b**). The *E:Z* ratio of the crude product (**3.5b**) was found to be >19:1, by <sup>1</sup>H NMR. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave **3.5b** (*E,E*) as a colourless oil (quantitative recovery). The <sup>13</sup>C NMR spectrum of **3.5b** (*E,E*) was identical to published data.<sup>3,24</sup>

Preparation of Methyl 2-butenate (3.5c)

The reaction was performed as described by the general procedure, both at -78 °C and at room temperature (r.t.), with 53 mg (1.2 mmol) of acetaldehyde (**3.3c**). Analysis, by <sup>1</sup>H NMR, of the reaction performed at -78 °C indicated the *E:Z* ratio of the crude product (**3.5c**) to be 8.0:1. Analysis, by <sup>1</sup>H NMR, of the reaction performed at r.t. indicated the *E:Z* ratio of the crude product (**3.5c**) to be 11:1. Purification by chromatography was not attempted due to the volatility of the product. The <sup>1</sup>H NMR spectrum of the product (**3.5c**) was identical to published data.<sup>3,25, 3.26</sup>

Preparation of Methyl 2-pentenoate (**3.5d**)

The reaction was performed as described by the general procedure, both at -78 °C and at r.t., with 70 mg (1.2 mmol) of propionaldehyde (**3.3d**). Analysis, by  $^1\text{H}$  NMR, of the reaction performed at -78 °C indicated the *E*:*Z* ratio of the crude product (**3.5d**) to be 3.6:1. Analysis, by  $^1\text{H}$  NMR, of the reaction performed at r.t. indicated the *E*:*Z* ratio of the crude product (**3.5d**) to be 7.0:1. Isolation was not attempted due to the volatility of the product. The  $^1\text{H}$  NMR spectrum of the product (**3.5d**) was identical to published data.<sup>3.27</sup>

Preparation of Methyl 2-nonenoate (**3.5e**)

The reaction was performed as described by the general procedure, both at -78 °C and at r.t., with 137 mg (1.2 mmol) of heptaldehyde (**3.3e**). Analysis, by  $^1\text{H}$  NMR, of the reaction performed at -78 °C indicated the *E*:*Z* ratio of the crude product (**3.5e**) to be 2.0:1. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z* as a colourless oil (180 mg, 81%). Analysis, by  $^1\text{H}$  NMR, of the reaction performed at r.t. indicated the *E*:*Z* ratio of the crude product (**3.5e**) to be 5.5:1. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z* as a colourless oil (164 mg, 74%). The  $^1\text{H}$  NMR spectrum of **3.5e** (*E*) was identical to published data.<sup>3.29</sup>

Spectral data for **3.5e** (*Z*) (from mixture):

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  6.23 (m, 1H,  $\text{COCHCH}$ ); 5.76 (dd, 1H,  $J=1.5, 11.3$  Hz,  $\text{COCH}$ ); 3.70 (s, 3H,  $\text{MeO}$ ); 2.64 (m, 2H,  $\text{CHCH}_2$ ); 1.28-1.43 (m, 8H,  $\text{Me}(\text{CH}_2)_4$ ); 0.87 (t, 3H,  $J=6.8$  Hz,  $\text{CH}_2\text{Me}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  166.6 ( $\text{CO}_2$ ); 150.8 ( $\text{COCHCH}$ ); 119.0 ( $\text{COCH}$ ); 50.7

(MeO); 31.5, 28.8, 22.4 (CH<sub>2</sub>); 13.8 (CH<sub>2</sub>Me). HRMS (on mixture) Calculated for C<sub>10</sub>H<sub>18</sub>O<sub>2</sub>: 170.13068. Found: 170.13040. FTIR (on mixture) (thin film) 2957; 2930; 2858; 1726; 1647 cm<sup>-1</sup>.

#### Preparation of Methyl 2-tridecenoate (3.5f)

The reaction was performed as described by the general procedure, both at -78 °C and at r.t., with 204 mg (1.2 mmol) of undecanal (**3.3f**). Analysis, by <sup>1</sup>H NMR, of the reaction performed at -78 °C indicated the *E*:*Z* ratio of the crude product (**3.5f**) to be 2.0:1.

Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z*, as a colourless oil (quantitative recovery). Analysis, by <sup>1</sup>H NMR, of the reaction performed at r.t. indicated the *E*:*Z* ratio of the crude product (**3.5f**) to be 5.7:1. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z*, as a colourless oil (quantitative recovery).

Spectral data:

**3.5f** (*E*) (from mixture). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.93 (dt, 1H, J=7.4, 15.6 Hz, COCHCH); 5.77 (d, 1H, J=15.6 Hz, COCH); 3.67 (s, 3H, MeO); 2.14 (q, 2H, J=7.3 Hz, CHCH<sub>2</sub>); 1.39 (s(br), 2H, CHCH<sub>2</sub>CH<sub>2</sub>); 1.21 (s(br), 14H, (CH<sub>2</sub>)<sub>7</sub>); 0.83 (t, 3H, J=6.3 Hz, CH<sub>2</sub>Me).

<sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 166.5 (CO<sub>2</sub>); 149.2 (COCHCH); 120.2 (COCH); 50.7 (MeO); 31.6, 31.3, 29.0, 28.9, 28.8, 28.7, 28.5, 27.4, 22.1 ((CH<sub>2</sub>)<sub>9</sub>); 13.5 (CH<sub>2</sub>Me). GCMS (*E* isomer) (m/z) 195 (22%, M<sup>+</sup>-OMe); 152 (23%, M<sup>+</sup>-MeCO<sub>2</sub>Me); 113 (37%); 87 (100%).

**3.5f** (*Z*) (from mixture). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.18 (dt, 1H, J=7.8, 11.2 Hz, COCHCH); 5.72 (d, 1H, J=11.8 Hz, COCH); 3.65 (s, 3H, MeO); 2.61 (m, 2H, CHCH<sub>2</sub>); 1.39 (s(br), 2H, CHCH<sub>2</sub>CH<sub>2</sub>); 1.21 (s(br), 14H, (CH<sub>2</sub>)<sub>7</sub>); 0.83 (t, 3H, J=6.3 Hz, CH<sub>2</sub>Me).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  166.1 ( $\text{CO}_2$ ); 150.4 ( $\text{COCHCH}$ ); 118.5 ( $\text{COCH}$ ); 50.3 ( $\text{MeO}$ ); 28.8, 28.4, 28.4 ( $\text{CH}_2$ ). Other signals were obscured by the *E* isomer. GCMS (*Z* isomer) ( $m/z$ ) 226 (7%,  $\text{M}^+$ ); 195 (10%,  $\text{M}^+ - \text{OMe}$ ); 183 (3%,  $\text{M}^+ - \text{COMe}$ ); 113 (100%). HRMS (on mixture) Calculated for  $\text{C}_{14}\text{H}_{26}\text{O}_2$ : 226.19328. Found: 226.19333. FTIR (on mixture) (thin film) 2926; 2855; 1728; 1647  $\text{cm}^{-1}$ .

#### Preparation of Methyl 4-methyl-2-pentenoate (**3.5g**)

The reaction was performed as described by the general procedure, both at  $-78\text{ }^\circ\text{C}$  and at r.t., with 87 mg (1.2 mmol) of isobutyraldehyde (**3.3g**). Analysis, by  $^1\text{H}$  NMR, of the reaction performed at  $-78\text{ }^\circ\text{C}$  indicated the *E:Z* ratio of the crude product (**3.5g**) to be 1:2.8. Analysis, by  $^1\text{H}$  NMR, of the reaction performed at r.t. indicated the *E:Z* ratio of the crude product (**3.5g**) to be 4.5:1. Purification by chromatography was not attempted due to the volatility of the product.

The reaction was also performed as described by the general procedure, using benzene in place of THF as the solvent. This reaction was performed at r.t., with 87 mg (1.2 mmol) of isobutyraldehyde (**3.3g**). The *E:Z* ratio of the crude product (**3.5g**) was found to be 10:1, by  $^1\text{H}$  NMR. Purification by chromatography was not attempted due to the volatility of the product.

The  $^1\text{H}$  NMR spectrum of **3.5g** was identical to published data.<sup>3,31</sup>

Preparation of Methyl 4-ethyl-2-hexenoate (**3.5h**)

The reaction was performed as described by the general procedure, both at -78 °C and at r.t., with 120 mg (1.2 mmol) of 2-ethylbutyraldehyde (**3.3h**). Analysis, by  $^1\text{H}$  NMR, of the reaction performed at -78 °C indicated the *E*:*Z* ratio of the crude product (**3.5h**) to be 1:5. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z*, as a colourless oil (128 mg, 68%). Analysis, by  $^1\text{H}$  NMR, of the reaction performed at r.t. indicated the *E*:*Z* ratio of the crude product (**3.5h**) to be 3.6:1. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z*, as a colourless oil (149 mg, 80%).

## Spectral data:

**3.5h** (*E*) (from mixture).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  6.73 (dd, 1H,  $J=8.8, 15.6$  Hz,  $\text{COCHCH}$ ); 5.78 (d, 1H,  $J=15.6$  Hz,  $\text{COCH}$ ); 3.72 (s, 3H, MeO); 1.94 (m, 1H,  $\text{CHCH}_2$ ); 1.32-1.48 (m, 4H,  $\text{CHCH}_2$ ); 0.83 (t, 6H,  $\text{CH}_2\text{Me}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  167.5 ( $\text{CO}_2$ ); 153.2 ( $\text{COCHCH}$ ); 120.7 ( $\text{COCH}$ ); 51.0 (MeO); 45.8 ( $\text{CH}_2\text{CH}$ ); 26.6 ( $\text{CH}_2$ ); 11.3 ( $\text{CH}_2\text{Me}$ ). GCMS (*E* isomer) ( $m/z$ ) 156 (13%,  $\text{M}^+$ ); 141 (18%,  $\text{M}^+-\text{Me}$ ); 124 (44%,  $\text{M}^+-\text{MeOH}$ ).

**3.5h** (*Z*) (from mixture).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  5.89 (m, 1H,  $\text{COCHCH}$ ); 5.81 (d, 1H,  $J=11.7$  Hz,  $\text{COCH}$ ); 3.67 (s, 3H, MeO); 3.26 (m, 1H,  $\text{CHCH}_2$ ); 1.44-1.52 (m, 4H,  $\text{CH}_2$ ); 0.84 (t, 6H,  $J=7.3$  Hz,  $\text{CH}_2\text{Me}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  166.0 ( $\text{CO}_2$ ); 154.9 ( $\text{COCHCH}$ ); 119.4 ( $\text{COCH}$ ); 50.5 (MeO); 40.9 ( $\text{CHCH}_2$ ); 27.5 ( $\text{CH}_2$ ); 11.3 ( $\text{CH}_2\text{Me}$ ). GCMS (*Z* isomer) ( $m/z$ ) 156 (44%,  $\text{M}^+$ ); 141 (46%,  $\text{M}^+-\text{Me}$ ); 127 (62%,  $\text{M}^+-\text{Et}$ ); 124 (33%,  $\text{M}^+-\text{MeOH}$ ).

HRMS (on mixture) Calculated for  $\text{C}_9\text{H}_{16}\text{O}_2$ : 156.11503. Found: 156.11547. FTIR (on mixture) (thin film) 2964; 2934; 2876; 1728; 1657  $\text{cm}^{-1}$ .



Preparation of Methyl 3-cyclohexyl-2-propenoate (**3.5i**)

The reaction was performed as described by the general procedure, both at -78 °C and at r.t., with 134 mg (1.2 mmol) of cyclohexanecarbaldehyde (**3.3i**). Analysis, by <sup>1</sup>H NMR, of the reaction performed at -78 °C indicated the *E*:*Z* ratio of the crude product (**3.5i**) to be 1:3.0. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z*, as a colourless oil (109 mg, 54%). Analysis, by <sup>1</sup>H NMR, of the reaction performed at r.t. indicated the *E*:*Z* ratio of the crude product (**3.5i**) to be 3.4:1. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z*, as a colourless oil (163 mg, 81%).

Spectral data:

**3.5i** (*E*) (from mixture). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.92 (dd, 1H, J=6.8, 17.4 Hz, COCHCH); 5.76 (d, 1H, J=17.4 Hz, COCH); 3.72. (s, 3H, MeO); 2.10 (m(br), 1H, CHCH<sub>2</sub>); 1.22-1.76 (m(br), 10H, (CH<sub>2</sub>)<sub>5</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 167.0 (CO<sub>2</sub>); 154.1 (COCHCH); 118.3 (COCH); 51.0 (MeO); 40.2 (CHCH<sub>2</sub>); 31.5, 25.7, 25.5 (CH<sub>2</sub>). GCMS (*E* isomer) (m/z) 168 (57%, M<sup>+</sup>); 136 (36%, M<sup>+</sup>-MeOH); 107 (24%); 94 (52%); 87 (84%).

**3.5i** (*Z*) (from mixture). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.03 (dd, 1H, J=9.8, 10.2 Hz, COCHCH); 5.66 (d, 1H, J=10.7 Hz, COCH); 3.69 (s, 3H, MeO); 3.29 (m(br), 1H, CHCH<sub>2</sub>); 1.05-1.76 (m(br), 10H, (CH<sub>2</sub>)<sub>5</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 166.5 (CO<sub>2</sub>); 155.7 (COCHCH); 117.0 (COCH); 50.7 (MeO); 37.1 (CHCH<sub>2</sub>); 32.2, 25.8, 25.3 (CH<sub>2</sub>). GCMS (*Z* isomer) (m/z) 168 (95% M<sup>+</sup>); 137 (28%, M<sup>+</sup>-MeO); 107 (32%); 94 (58%); 87 (78%).

HRMS (on mixture) Calculated for C<sub>10</sub>H<sub>16</sub>O<sub>2</sub>: 168.11503. Found: 168.11518. FTIR (on mixture) (thin film) 2828; 2853; 1726; 1652 cm<sup>-1</sup>.

Preparation of Methyl 4-methyl-2-tridecenoate (**3.5j**)

The reaction was performed as described by the general procedure, both at -78 °C and at r.t., with 221 mg (1.2 mmol) of 2-methylundecanal (**3.3j**). Analysis, by  $^1\text{H}$  NMR, of the reaction performed at -78 °C indicated the *E*:*Z* ratio of the crude product (**3.5j**) to be 1:2.8. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave **3.5j** (*E*) (60 mg, 21%) and **3.5j** (*Z*) (179 mg, 62%), both as colourless oils. Analysis, by  $^1\text{H}$  NMR, of the reaction performed at r.t. indicated the *E*:*Z* ratio of the crude product (**3.5j**) to be 3.8:1. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave **3.5j** (*E*) (214 mg, 74%) and **3.5j** (*Z*) (56 mg, 20%) both as colourless oils.

Spectral data:

**3.5j** (*E*).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  6.86 (dd, 1H,  $J=7.8, 15.2$  Hz,  $\text{COCHCH}$ ); 5.77 (dd, 1H,  $J=1.4, 15.6$  Hz,  $\text{COCH}$ ); 3.72 (s, 3H, MeO); 2.27 (m, 1H,  $\text{CHMe}$ ); 1.24 (m, 16H,  $(\text{CH}_2)_8$ ); 1.03 (d, 3H,  $J=6.3$  Hz,  $\text{CHMe}$ ); 0.87 (t, 3H,  $J=7.3$  Hz,  $\text{CH}_2\text{Me}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  167.2 ( $\text{CO}_2$ ); 154.9 ( $\text{COCHCH}$ ); 119.0 ( $\text{COCH}$ ); 51.2 (MeO); 36.4 ( $\text{CHMe}$ ); 35.9, 31.8, 29.5, 29.5, 29.4, 29.2, 27.1, 22.5 ( $(\text{CH}_2)_8$ ); 19.3 ( $\text{CHMe}$ ); 14.0 ( $\text{CH}_2\text{Me}$ ). HRMS. Calculated for  $\text{C}_{15}\text{H}_{28}\text{O}_2$ : 240.20893. Found: 240.20900. MS ( $m/z$ ) 240 (46%,  $\text{M}^+$ ); 209 (20%,  $\text{M}^+-\text{MeOH}$ ); 166 (13%); 128 (100%). FTIR (thin film) 2956; 2926; 2855; 1728; 1657  $\text{cm}^{-1}$ .

**3.5j** (*Z*).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  5.97 (dd, 1H,  $J=10.2, 11.3$  Hz,  $\text{COCHCH}$ ); 5.70 (dd, 1H,  $J=0.9, 10.8$  Hz,  $\text{COCH}$ ); 3.70 (s, 3H, MeO); 3.45 (m, 1H,  $\text{CHMe}$ ); 1.24 (m, 16H,  $(\text{CH}_2)_8$ ); 0.99 (d, 3H,  $J=6.8$  Hz,  $\text{CHMe}$ ); 0.87 (t, 3H,  $J=7.3$  Hz,  $\text{CH}_2\text{Me}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  166.4 ( $\text{CO}_2$ ); 156.3 ( $\text{COCHCH}$ ); 117.6 ( $\text{COCH}$ ); 50.6 (MeO); 36.9 ( $\text{CHMe}$ ); 32.5, 31.8, 29.6, 29.4, 29.4, 29.2, 27.2, 22.5 ( $(\text{CH}_2)_8$ ); 19.3 ( $\text{CHMe}$ ); 14.0 ( $\text{CH}_2\text{Me}$ ). HRMS. Calculated for  $\text{C}_{15}\text{H}_{28}\text{O}_2$ : 240.20893. Found: 240.20868. MS ( $m/z$ ) 240 (66%,  $\text{M}^+$ ); 212

(24%, M<sup>+</sup>-CO); 183 (6%, M<sup>+</sup>-CHCO<sub>2</sub>); 127 (100%). FTIR (thin film) 2926; 2855; 1728; 1647 cm<sup>-1</sup>.

#### General Procedure<sup>3,7</sup> for Reactions with Triethyl phosphonoacetate (**3.1b**)

A stirring solution of 250 mg (1.1 mmol) of triethyl phosphonoacetate (**3.1b**) in 2.5 ml of dry THF was cooled to 0 °C under a nitrogen atmosphere and 0.68 ml (1.1 mmol) of a 1.6 M solution of *n*-BuLi in hexane was added dropwise. After 10 minutes, the reaction was brought to the desired temperature and the aldehyde (0.92 mmol) was added dropwise. The resultant mixture was stirred for 1.5 hours, quenched by the addition of 3.0 ml of water, and further diluted with 5.0 ml of ether. The aqueous layer was removed and the organic layer was washed with saturated brine, dried with MgSO<sub>4</sub>, filtered, and the solvent was evaporated under reduced pressure. The crude product was analysed directly by <sup>1</sup>H NMR and where indicated, purified by radial chromatography on silica (80% petroleum ether, 20% ethyl acetate).

#### Preparation of Ethyl cinnamate (**3.6a**)

The reaction was performed as described by the general procedure, with 98 mg (0.92 mmol) of benzaldehyde (**3.3a**). The *E*:*Z* ratio of the crude product (**3.6a**) was found to be >19:1, by <sup>1</sup>H NMR. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave **3.6a** (*E*) as a colourless oil (180 mg, 85%). The <sup>1</sup>H NMR spectrum of **3.6a** (*E*) was identical to published data.<sup>3,23</sup>

Preparation of Ethyl 2-pentenoate (**3.6d**)

The reaction was performed as described by the general procedure, both at -78 °C and at r.t., with 53 mg (0.92 mmol) of propionaldehyde (**3.3d**). Analysis, by  $^1\text{H}$  NMR, of the reaction performed at -78 °C indicated the *E*:*Z* ratio of the crude product (**3.6d**) to be 9.3:1. Analysis, by  $^1\text{H}$  NMR, of the reaction performed at r.t. indicated the *E*:*Z* ratio of the crude product (**3.6d**) to be 19:1. Purification by chromatography was not attempted due to the volatility of the product. The  $^1\text{H}$  NMR spectrum of the product (**3.6d**) was identical to published data.<sup>3.28</sup>

Preparation of Ethyl 2-nonenoate (**3.6e**)

The reaction was performed as described by the general procedure, both at -78 °C and at r.t., with 126 mg (0.92 mmol) of heptaldehyde (**3.3e**). Analysis, by  $^1\text{H}$  NMR, of the reaction performed at -78 °C indicated the *E*:*Z* ratio of the crude product (**3.6e**) to be 9.4:1. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z* as a colourless oil (112 mg, 55%). Analysis, by  $^1\text{H}$  NMR, of the reaction performed at r.t. indicated the *E*:*Z* ratio of the crude product (**3.6e**) to be >19:1. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave **3.6e** (*E*) as a colourless oil (132 mg, 65%). The  $^1\text{H}$  NMR spectrum of **3.6e** (*E*) was identical to published data.<sup>3.30</sup>

Spectral data for **3.6e** (*Z*) (from mixture):

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  6.21 (dt, 1H,  $J=7.8, 11.3$  Hz,  $\text{COCHCH}$ ); 5.74 (d, 1H,  $J=11.3$  Hz,  $\text{COCH}$ ); 2.62 (m, 2H,  $\text{CHCH}_2$ ); 0.95 (t, 3H,  $J=7.3$  Hz,  $\text{MeCH}_2\text{CO}$ ). Other signals were obscured by the *E* isomer.  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  165.8 ( $\text{CO}_2$ ); 149.9 ( $\text{COCHCH}$ ); 119.3

(COCH); 59.1 (CH<sub>2</sub>O). Other signals were obscured by the *E* isomer. HRMS (on mixture) Calculated for C<sub>11</sub>H<sub>20</sub>O<sub>2</sub>: 184.14633. Found: 184.14646. FTIR (on mixture) (thin film)  $\lambda$  2929; 2858; 2256; 1705; 1652 cm<sup>-1</sup>.

#### Preparation of Ethyl 4-methyl-2-pentenoate (3.6g)

The reaction was performed as described by the general procedure, both at -78 °C and at r.t., with 66 mg (0.92 mmol) of isobutyraldehyde (**3.3g**). Analysis, by <sup>1</sup>H NMR, of the reaction performed at -78 °C indicated the *E*:*Z* ratio of the crude product (**3.6g**) to be 1.5:1. Analysis, by <sup>1</sup>H NMR, of the reaction performed at r.t. indicated the *E*:*Z* ratio of the crude product (**3.6g**) to be 13:1. Purification by chromatography was not attempted due to the volatility of the product.

The reaction was also performed as described by the general procedure, using benzene in place of THF as the solvent. This reaction was performed at r.t. with 66 mg (0.92 mmol) of isobutyraldehyde (**3.3g**), and the *E*:*Z* ratio of the crude product (**3.6g**) was found to be >19:1, by <sup>1</sup>H NMR. Purification by chromatography was not attempted due to the volatility of the product.

The <sup>1</sup>H NMR spectrum of the product (**3.6g**) was identical to published data.<sup>3,28</sup>

#### Preparation of Ethyl 4-ethyl-2-hexenoate (3.6h)

The reaction was performed as described by the general procedure, both at -78 °C and at r.t., with 92 mg (0.92 mmol) of 2-ethylbutyraldehyde (**3.3h**). Analysis, by <sup>1</sup>H NMR, of the reaction performed at -78 °C indicated the *E*:*Z* ratio of the crude product (**3.6h**) to be 1.5:1. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave

an inseparable mixture of *E* and *Z*, as a colourless oil (123 mg, 78%). Analysis, by  $^1\text{H}$  NMR, of the reaction performed at r.t. indicated the *E:Z* ratio of the crude product (**3.6h**) to be 12:1. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z*, as a colourless oil (144 mg, 92%). The  $^1\text{H}$  NMR spectrum of the product (**3.6h**) was identical to published data.<sup>3.32</sup>

#### General Procedure<sup>3.7</sup> for Reactions with Methyl diphenylphosphonoacetate (**3.1c**)

A stirring solution of 250 mg (0.82 mmol) of methyl diphenylphosphonoacetate (**3.1c**) in 2.5 ml of dry THF was cooled to 0 °C under a nitrogen atmosphere and 0.51 ml (0.82 mmol) of a 1.6 M solution of *n*-BuLi in hexane was added dropwise. After 10 minutes, the reaction was cooled to -78 °C and the aldehyde (0.68 mmol) was added dropwise. The resultant mixture was stirred for 1.5 hours, quenched by the addition of 3.0 ml of water, and further diluted with 5.0 ml of ether. The aqueous layer was removed and the organic layer was washed with saturated brine, dried with  $\text{MgSO}_4$ , filtered, and the solvent was evaporated under reduced pressure. The crude product was analysed directly by  $^1\text{H}$  NMR and where indicated, purified by radial chromatography on silica (80% petroleum ether, 20% ethyl acetate).

#### Preparation of Methyl cinnamate (**3.5a**)

The reaction was performed as described by the general procedure, with 72 mg (0.68 mmol) of benzaldehyde (**3.3a**). The *E:Z* ratio of the crude product (**3.5a**) was found to be 1:2.2, by  $^1\text{H}$  NMR. Isolation by radial chromatography (80% petroleum ether, 20%

ethyl acetate) gave an inseparable mixture of *E* and *Z* as a colourless oil (69 mg, 63%).

Spectral data for **3.5a** was identical to published data.<sup>3.21, 3.22</sup>

#### Preparation of Methyl 2-pentenoate (**3.5d**)

The reaction was performed as described by the general procedure, with 53 mg (0.68 mmol) of propionaldehyde (**3.3d**). The *E:Z* ratio of the crude product (**3.5d**) was found to be 1:13, by <sup>1</sup>H NMR. Purification by chromatography was not attempted due to the volatility of the product. The <sup>1</sup>H NMR spectrum of the product (**3.5d**) was identical to published data.<sup>3.27</sup>

#### Preparation of Methyl 2-nonenoate (**3.5e**)

The reaction was performed as described by the general procedure, with 78 mg (0.68 mmol) of heptaldehyde (**3.3e**). The *E:Z* ratio of the crude product (**3.5e**) was found to be 1:7.0, by <sup>1</sup>H NMR. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z* as a colourless oil (67 mg, 58%). The <sup>1</sup>H NMR spectrum of **3.5e** (*E*) was identical to published data.<sup>3.29</sup> See section “Reactions with Trimethyl phosphonoacetate” for spectral data on **3.5e** (*Z*).

#### Preparation of Methyl 4-methyl-2-pentenoate (**3.5g**)

The reaction was performed as described by the general procedure, with 49 mg (0.68 mmol) of isobutyraldehyde (**3.3g**). The *E:Z* ratio of the crude product (**3.5g**) was found to be 1:3.3, by <sup>1</sup>H NMR. Purification by chromatography was not attempted due to

the volatility of the product. The  $^1\text{H}$  NMR spectrum of **3.5g** was identical to published data.<sup>3.31</sup>

#### Preparation of Methyl 4-ethyl-2-hexenoate (**3.5h**)

The reaction was performed as described by the general procedure, with 34 mg (0.68 mmol) of 2-ethylbutyraldehyde (**3.3h**). The *E*:*Z* ratio of the crude product (**3.5h**) was found to be 1:3.8, by  $^1\text{H}$  NMR. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z*, as a colourless oil (42 mg, 72%). See section “Reactions with Trimethyl phosphonoacetate” for spectral data.

#### Preparation of Methyl 4-methyl-2-tridecenoate (**3.5j**)

The reaction was performed as described by the general procedure, with 63 mg (0.68 mmol) of 2-methylundecanal (**3.3j**). The *E*:*Z* ratio of the crude product (**3.5j**) was found to be 1:5.7, by  $^1\text{H}$  NMR. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave **3.5j** (*E*) (9 mg, 11%) and **3.5j** (*Z*) (52 mg, 60%), both as colourless oils. See section “Reactions with Trimethyl phosphonoacetate” for spectral data.

#### General Procedure<sup>3.7</sup> for Reactions with Ethyl dimethylphosphonoacetate (**3.1d**)

A stirring solution of 250 mg (1.3 mmol) of ethyl dimethylphosphonoacetate (**3.1d**) in 2.5 ml of dry THF was cooled to 0 °C under a nitrogen atmosphere and 0.81 ml



(1.3 mmol) of a 1.6 M solution of *n*-BuLi in hexane was added dropwise. After 10 minutes, the reaction was cooled to -78 °C and the aldehyde (1.1 mmol) was added dropwise. The resultant mixture was stirred for 1.5 hours, quenched by the addition of 3.0 ml of water, and further diluted with 5.0 ml of ether. The aqueous layer was removed and the organic layer was washed with saturated brine, dried with MgSO<sub>4</sub>, filtered, and the solvent was evaporated under reduced pressure. The crude product was analysed directly by <sup>1</sup>H NMR and where indicated, purified by radial chromatography on silica (80% petroleum ether, 20% ethyl acetate).

#### Preparation of Ethyl cinnamate (3.6a)

The reaction was performed as described by the general procedure, with 117 mg (1.1 mmol) of benzaldehyde (**3.3a**). The *E*:*Z* ratio of the crude product (**3.6a**) was found to be >19:1, by <sup>1</sup>H NMR. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave **3.6a** (*E*) as a colourless oil (quantitative recovery). The <sup>1</sup>H NMR spectrum of **3.6a** (*E*) was identical to published data.<sup>3,23</sup>

#### Preparation of Ethyl 2-nonenolate (3.6e)

The reaction was performed as described by the general procedure, with 126 mg (1.1 mmol) of heptaldehyde (**3.3e**). The *E*:*Z* ratio of the crude product (**3.6e**) was found to be 1.2:1, by <sup>1</sup>H NMR. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z* as a colourless oil (quantitative recovery). The <sup>1</sup>H NMR spectrum **3.6e** (*E*) was identical to published data.<sup>3,30</sup> See section "Reactions with Triethyl phosphonoacetate" for spectral data on **3.6e** (*Z*).

Preparation of Ethyl 4-methyl-2-pentenoate (3.6g)

The reaction was performed as described by the general procedure, with 79 mg (1.1 mmol) of isobutyraldehyde (3.3g). The *E:Z* ratio of the crude product (3.6g) was found to be 1:2.9, by  $^1\text{H}$  NMR. Purification by chromatography was not attempted due to the volatility of the product. The  $^1\text{H}$  NMR spectrum of the product (3.6g) was identical to published data.<sup>3,28</sup>

General Procedure<sup>3,7</sup> for Reactions with Methyl diethylphosphonoacetate (3.1e)

A stirring solution of 250 mg (1.2 mmol) of methyl diethylphosphonoacetate (3.1e) in 2.5 ml of dry THF was cooled to 0 °C under a nitrogen atmosphere and 0.75 ml (1.2 mmol) of a 1.6 M solution of *n*-BuLi in hexane was added dropwise. After 10 minutes, the reaction was cooled to -78 °C and the aldehyde (1.0 mmol) was added dropwise. The resultant mixture was stirred for 1.5 hours, quenched by the addition of 3.0 ml of water, and further diluted with 5.0 ml of ether. The aqueous layer was removed and the organic layer was washed with saturated brine, dried with  $\text{MgSO}_4$ , filtered, and the solvent was evaporated under reduced pressure. The crude product was analysed directly by  $^1\text{H}$  NMR and where indicated, purified by radial chromatography on silica (80% petroleum ether, 20% ethyl acetate).

Preparation of Methyl cinnamate (3.5a)

The reaction was performed as described by the general procedure, with 106 mg (1.0 mmol) of benzaldehyde (3.3a). The *E:Z* ratio of the crude product (3.5a) was found

to be >19:1, by  $^1\text{H}$  NMR. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave **3.5a** (*E*) as a colourless oil (104 mg, 64%). The FTIR spectrum of **3.5a** (*E*) was identical to published data.<sup>3.21</sup>

#### Preparation of Methyl 2-nonenoate (**3.5e**)

The reaction was performed as described by the general procedure, with 114 mg (1.0 mmol) of heptaldehyde (**3.3e**). The *E*:*Z* ratio of the crude product (**3.5e**) was found to be 6.7:1, by  $^1\text{H}$  NMR. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z* as a colourless oil (115 mg, 68%). The  $^1\text{H}$  NMR spectrum of **3.5e** (*E*) was identical to published data.<sup>3.29</sup> See section “Reactions with Trimethyl phosphonoacetate” for spectral data on **3.5e** (*Z*).

#### Preparation of Methyl 4-methyl-2-pentenoate (**3.5g**)

The reaction was performed as described by the general procedure, with 86 mg (1.0 mmol) of isobutyraldehyde (**3.3g**). The *E*:*Z* ratio of the crude product (**3.5g**) was found to be 2.4:1, by  $^1\text{H}$  NMR. Purification by chromatography was not attempted due to the volatility of the product. The  $^1\text{H}$  NMR spectrum of **3.5g** was identical to published data.<sup>3.31</sup>

#### General Procedure<sup>3.7</sup> for Reactions with Methyl diisopropylphosphonoacetate (**3.1f**)

A stirring solution of 33 mg (0.14 mmol) of methyl diisopropylphosphonoacetate (**3.1f**) in 0.5 ml of dry THF was cooled to 0 °C under a nitrogen atmosphere and 0.09 ml

(0.14 mmol) of a 1.6 M solution of *n*-BuLi in hexane was added dropwise. After 10 minutes, the reaction was cooled to -78 °C and the aldehyde (0.12 mmol) was added dropwise. The resultant mixture was stirred for 1.5 hours, quenched by the addition of 0.5 ml of water, and further diluted with 1.0 ml of ether. The aqueous layer was removed and the organic layer was washed with saturated brine, dried with MgSO<sub>4</sub>, filtered, and the solvent was evaporated under reduced pressure. The crude product was analysed directly by <sup>1</sup>H NMR and where indicated, purified by radial chromatography on silica (80% petroleum ether, 20% ethyl acetate).

#### Preparation of Methyl cinnamate (3.5a)

The reaction was performed as described by the general procedure, with 12 mg (0.12 mmol) of benzaldehyde (**3.3a**). The *E*:*Z* ratio of the crude product (**3.5a**) was found to be >19:1, by <sup>1</sup>H NMR. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave **3.5a** (*E*) as a colourless oil (5 mg, 26%). The FTIR spectrum of **3.5a** (*E*) was identical to published data.<sup>3,21</sup>

#### Preparation of Methyl 2-nonenoate (3.5e)

The reaction was performed as described by the general procedure, with 13 mg (0.12 mmol) of heptaldehyde (**3.3e**). The *E*:*Z* ratio of the crude product (**3.5e**) was found to be >19:1, by <sup>1</sup>H NMR. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave **3.5e** (*E*) as a colourless oil (7 mg, 35%). The <sup>1</sup>H NMR spectrum of **3.5e** (*E*) was identical to published data.<sup>3,29</sup>

Preparation of Methyl 4-methyl-2-pentenoate (**3.5g**)

The reaction was performed as described by the general procedure, with 8 mg (0.12 mmol) of isobutyraldehyde (**3.3g**). The *E:Z* ratio of the crude product (**3.5g**) was found to be 10:1, by  $^1\text{H}$  NMR. Purification by chromatography was not attempted due to the volatility of the product. The  $^1\text{H}$  NMR spectrum of **3.5g** was identical to published data.<sup>3,31</sup>

General Procedure<sup>3,7</sup> for Reactions with Methyl di-(4-methoxyphenyl)-phosphonoacetate (**3.1g**)

A stirring solution of 250 mg (0.65 mmol) of methyl di-(4-methoxyphenyl)-phosphonoacetate (**3.1g**) in 2.5 ml of dry THF was cooled to 0 °C under a nitrogen atmosphere and 0.41 ml (0.65 mmol) of a 1.6 M solution of *n*-BuLi in hexane was added dropwise. After 10 minutes, the reaction was cooled to -78 °C and the aldehyde (0.54 mmol) was added dropwise. The resultant mixture was stirred for 1.5 hours, quenched by the addition of 3.0 ml of water, and further diluted with 5.0 ml of ether. The aqueous layer was removed and the organic layer was washed with saturated brine, dried with  $\text{MgSO}_4$ , filtered, and the solvent was evaporated under reduced pressure. The crude product was analysed directly by  $^1\text{H}$  NMR and where indicated, purified by radial chromatography on silica (80% petroleum ether, 20% ethyl acetate).

### Preparation of Methyl cinnamate (3.5a)

The reaction was performed as described by the general procedure, with 57 mg (0.54 mmol) of benzaldehyde (**3.3a**). The *E*:*Z* ratio of the crude product (**3.5a**) was found to be 1:1.3, by <sup>1</sup>H NMR. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z*, as a colourless oil (74 mg, 85%). Spectral data for **3.5a** was identical to published data.<sup>3.21, 3.22</sup>

### Preparation of Methyl 2-nonenolate (3.5e)

The reaction was performed as described by the general procedure, with 62 mg (0.54 mmol) of heptaldehyde (**3.3e**). The *E*:*Z* ratio of the crude product (**3.5e**) was found to be 1:4.2, by <sup>1</sup>H NMR. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z* as a colourless oil (68 mg, 75%). The <sup>1</sup>H NMR spectrum of **3.5e** (*E*) was identical to published data.<sup>3.29</sup> See section “Reactions with Trimethyl phosphonoacetate” for spectral data on **3.5e** (*Z*).

### Preparation of Methyl 4-methyl-2-pentenoate (3.5g)

The reaction was performed as described by the general procedure, with 39 mg (0.54 mmol) of isobutyraldehyde (**3.3g**). The *E*:*Z* ratio of the crude product (**3.5g**) was found to be 1:2.1, by <sup>1</sup>H NMR. Purification by chromatography was not attempted due to the volatility of the product. The <sup>1</sup>H NMR spectrum of **3.5g** was identical to published data.<sup>3.31</sup>

General Procedure<sup>3.7</sup> for Reactions with Methyl di-(3-methoxyphenyl)-  
phosphonoacetate (**3.1h**)

A stirring solution of 125 mg (0.34 mmol) of methyl di-(3-methoxyphenyl)-phosphonoacetate (**3.1h**) in 1.3 ml of dry THF was cooled to 0 °C under a nitrogen atmosphere and 0.21 ml (0.34 mmol) of a 1.6 M solution of *n*-BuLi in hexane was added dropwise. After 10 minutes, the reaction was cooled to -78 °C and the aldehyde (0.28 mmol) was added dropwise. The resultant mixture was stirred for 1.5 hours, quenched by the addition of 1.5 ml of water, and further diluted with 2.5 ml of ether. The aqueous layer was removed and the organic layer was washed with saturated brine, dried with MgSO<sub>4</sub>, filtered, and the solvent was evaporated under reduced pressure. The crude product was analysed directly by <sup>1</sup>H NMR and where indicated, purified by radial chromatography on silica (80% petroleum ether, 20% ethyl acetate).

Preparation of Methyl cinnamate (**3.5a**)

The reaction was performed as described by the general procedure, with 30 mg (0.28 mmol) of benzaldehyde (**3.3a**). The *E*:*Z* ratio of the crude product (**3.5a**) was found to be 1:2.4, by <sup>1</sup>H NMR. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z*, as a colourless oil (quantitative recovery). Spectral data for **3.5a** was identical to published data.<sup>3.21, 3.22</sup>

Preparation of Methyl 2-nonenoate (**3.5e**)

The reaction was performed as described by the general procedure, with 32 mg (0.28 mmol) of heptaldehyde (**3.3e**). The *E*:*Z* ratio of the crude product (**3.5e**) was found to be 1:7.2, by  $^1\text{H}$  NMR. Isolation by radial chromatography (80% petroleum ether, 20% ethyl acetate) gave an inseparable mixture of *E* and *Z* as a colourless oil (28 mg, 58%).

The  $^1\text{H}$  NMR spectrum of **3.5e** (*E*) was identical to published data.<sup>3.29</sup> See section “Reactions with Trimethyl phosphonoacetate” for spectral data on **3.5e** (*Z*).

Preparation of Methyl 4-methyl-2-pentenoate (**3.5g**)

The reaction was performed as described by the general procedure, with 20 mg (0.28 mmol) of isobutyraldehyde (**3.3g**). The *E*:*Z* ratio of the crude product (**3.5g**) was found to be 1:3.3, by  $^1\text{H}$  NMR. Purification by chromatography was not attempted due to the volatility of the product. The  $^1\text{H}$  NMR spectrum of **3.5g** was identical to published data.<sup>3.31</sup>

General Procedure<sup>3.19</sup> for the Preparation of the Phosphonates (**3.1c**, **3.1f**, **3.1g**, and **3.1h**)

## Step 1

Trimethyl phosphonoacetate (**3.1a**) (5.000 g, 27 mmol) was cooled to 0 °C and phosphorous pentachloride (14.000 g, 54 mmol) was added. The resultant mixture was heated to 75 °C for 10 hours and then distilled at 105-110 °C (2mm Hg) to yield the intermediate ( $\text{Cl}_2\text{POCH}_2\text{CO}_2\text{Me}$ ) (5.100 g, 98%).



## Step 2

To the intermediate ( $\text{Cl}_2\text{POCH}_2\text{CO}_2\text{Me}$ ) (1.000 g, 5.2 mmol) in 5.0 ml of benzene, at 0 °C, was added a solution of the alcohol (10.4 mmol) and  $\text{Et}_3\text{N}$  (126 mg, 12.5 mmol), also in benzene (2.0 ml). After stirring at r.t., the mixture was diluted with ethyl acetate, filtered, and the solvent was evaporated from the filtrate under reduced pressure.

Preparation of Methyl diphenylphosphonoacetate (3.1c)

The preparation was performed as described by the general procedure, with 979 mg (10.4 mmol) of phenol. The reaction mixture was stirred for 1 hour. The product (**3.1c**) was purified by radial chromatography (50% petroleum ether, 50% ethyl acetate) to give a colourless oil (1.15g, 72%).

Spectral data for **3.1c**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.19-7.36 (m, 10H, ArH); 3.76 (s, 3H, Me); 3.28 (d, 2H,  $J=22.0$  Hz,  $\text{CH}_2$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 164.9 (d,  $J=6.1$  Hz,  $\text{CO}_2$ ); 149.7 (d,  $J=9.1$  Hz), 129.6, 125.3, 120.4, (Ar); 52.5 (OMe); 33.5 (d,  $J=138.0$  Hz,  $\text{CH}_2$ ). HRMS. Calculated for  $\text{C}_{15}\text{H}_{15}\text{O}_5\text{P}$ : 306.06571. Found: 306.06562. FTIR (thin film) 2945; 1744; 1591  $\text{cm}^{-1}$ .

Preparation of Methyl diisopropylphosphonoacetate (3.1f)

The preparation was performed as described by the general procedure, with 625 mg (10.4 mmol) of 2-propanol. The reaction mixture was stirred for 24 hours. The product (**3.1f**) was purified by radial chromatography (50% petroleum ether, 50% ethyl acetate) to give a colourless oil (114 mg, 9%).

Spectral data for **3.1f**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  4.75 (m, 2H, CH); 3.72 (s, 3H, OMe); 2.93 (d, 2H,  $J=21.5$  Hz,  $\text{CH}_2$ ); 1.33 (d, 12H,  $J=5.9$  Hz, ( $\text{Me}_2\text{CH}$ )).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 162.3 ( $\text{CO}_2$ ); 71.6 (d,  $J=7.3$  Hz, CH); 52.4 (OMe); 35.3 (d,  $J=135.6$  Hz,  $\text{CH}_2$ ); 24.0, 23.8, 23.7, 21.7 ( $\text{Me}_2\text{CH}$ ). HRMS. Calculated for  $\text{C}_8\text{H}_{16}\text{O}_5\text{P}$  ( $\text{M}^+-\text{Me}$ ): 223.07354. Found: 223.07317. FTIR (thin film) 2981; 1726; 1574  $\text{cm}^{-1}$ .

#### Preparation of Methyl di-(4-methoxyphenyl)phosphonoacetate (**3.1g**)

The preparation was performed as described by the general procedure, with 1.290 g (10.4 mmol) of 4-methoxyphenol. The reaction mixture was stirred for 1 hour. The product (**3.1g**) was purified by radial chromatography (50% petroleum ether, 50% ethyl acetate) to give a colourless oil (1.240 g, 75%).

Spectral data for **3.1g**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.14 (d, 4H,  $J=9.3$  Hz, ArH); 6.84 (d, 4H,  $J=9.3$  Hz, ArH); 3.77 (s, 9H, Me); 3.23 (d, 2H,  $J=21.5$  Hz,  $\text{CH}_2$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  165.2 (d,  $J=6.2$  Hz,  $\text{CO}_2$ ); 156.9, 143.3 (d,  $J=8.3$  Hz), 121.3, 114.5 (Ar); 55.2 ( $\text{MeOC}_4\text{H}_6$ ); 52.5 ( $\text{CO}_2\text{Me}$ ); 33.2 (d,  $J=136.7$  Hz,  $\text{CH}_2$ ). HRMS. Calculated for  $\text{C}_{17}\text{H}_{19}\text{O}_7\text{P}$ : 366.08684. Found: 366.08602. FTIR (thin film) 3479; 3003; 2955; 2012; 2839; 1744; 1595  $\text{cm}^{-1}$ .

#### Preparation of Methyl di-(3-methoxyphenyl)phosphonoacetate (**3.1h**)

The preparation was performed as described by the general procedure, with 1.290 g (10.4 mmol) of 3-methoxyphenol. The reaction mixture was stirred for 1 hour. The

product (**3.1h**) was purified by radial chromatography (50% petroleum ether, 50% ethyl acetate) to give a colourless oil (983 mg, 52%).

Spectral data for **3.1h**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.22 (m, 2H, ArH); 6.77 (m, 6H, ArH); 3.76 (s, 9H, Me); 3.27 (d, 2H,  $J=21.5$  Hz,  $\text{CH}_2$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  165.2 (d,  $J=6.3$  Hz,  $\text{CO}_2$ ); 160.7, 150.8 (d,  $J=8.3$  Hz), 130.1, 112.6, 111.5, 106.6 (Ar); 55.4 ( $\text{MeOC}_4\text{H}_6$ ); 52.8 ( $\text{CO}_2\text{Me}$ ); 33.7 (d,  $J=140.8$  Hz,  $\text{CH}_2$ ). HRMS. Calculated for  $\text{C}_{17}\text{H}_{19}\text{O}_7\text{P}$ : 366.08684. Found: 366.08710. FTIR (thin film) 3477; 3078; 3005; 2954; 2837; 2247; 1744; 1609; 1589  $\text{cm}^{-1}$ .

## References

- 3.1. Boutagy, J. Thomas, R. *Chem. Rev.* **1974**, 74 (1), 87 and references therein.
- 3.2. Maryanoff, B. Reitz, A. *Chem. Rev.* **1989**, 89 (4), 863.
- 3.3. Wolinsky, J. Erickson, K. *J. Org. Chem.* **1965**, 30, 2208.
- 3.4. Walker, B. In: *Organophosphorous Reagents in Organic Synthesis*. Editor: Cadogan, J. Academic Press, London. **1979**, 25, 155 and references therein.
- 3.5. Wadsworth, W. In: *Organic Reactions*. Editor: Dauben, W. John Wiley & Sons, Inc., New York. **1977**, 25, 73 and references therein.
- 3.6. Lefebvre, G. Seyden-Penne, J. *J. Chem. Soc. Chem. Comm.* **1970**, 1308.
- 3.7. Thompson, S. Heathcock, C. *J. Org. Chem.* **1990**, 55, 3386.
- 3.8. Larson, R. Aksnes, G. *Phosphorus and Sulfur*. **1983**, 16, 339 and references therein.
- 3.9. Deschamps, B. Lampin, J. Mathey, F. Seyden-Penne, J. *Tetrahedron Lett.* **1977**, 13, 1137.
- 3.10. Breuer, E. Bannet, D. *Tetrahedron Lett.* **1977**, 13, 1141.
- 3.11. Kinstle, T. Mandanas, B. *J. Chem. Soc. Chem. Comm.* **1968**, 1699.
- 3.12. Etemad-Moghadam, G. Seyden-Penne, J. *Tetrahedron*. **1984**, 40 (24), 5153.
- 3.13. Nagaoka, H. Kishi, Y. *Tetrahedron*. **1981**, 37 (23), 3873.
- 3.14. Popoff, I. Dever, J. Leader, G. *J. Org. Chem.* **1969**, 34 (4), 1128.
- 3.15. Bernardi, A. Cardani, S. Scolastico, C. Villa, R. *Tetrahedron*. **1988**, 44 (2), 491.
- 3.16. Still, W. Gennari, C. *Tetrahedron Lett.* **1983**, 24 (41), 4405.
- 3.17. Wadsworth, D. Schupp, O. Seus, E. Ford, J. *J. Org. Chem.* **1965**, 30, 680.
- 3.18. Minami, N. Sung Ko, S. Kishi, Y. *J. Am. Chem. Soc.* **1982**, 104, 1109.
- 3.19. Ando, K. *Tetrahedron Lett.* **1995**, 36 (23), 4105.

- 3.20. Reddy, A. Gopal, V. Rao, V. *Indian. J. Chem.* **1996**, 35B, 312.
- 3.21. Pouchet, C. *The Aldrich Library of FT-IR Spectra*. 1st ed. Aldrich Chemical Company, Inc., Wisconsin. **1985**, 3, 1354D.
- 3.22. Jacobsen, E. Deng, L. Furukawa, Y. Martinez, L. *Tetrahedron*. **1994**, 50 (15), 4323.
- 3.23. Pouchet, C. *The Aldrich Library of NMR Spectra*. 2nd ed. Aldrich Chemical Company, Inc., Wisconsin. **1983**, 2, 276B.
- 3.24. Rodriguez, J. Waegell, B. *Syn.* **1988**, 7, 534.
- 3.25. Pouchet, C. *The Aldrich Library of NMR Spectra*. 2nd. ed. Aldrich Chemical Company, Inc., Wisconsin. **1983**, 1, 504D.
- 3.26. Orfanopoulos, M. Smonou, I. Foote, C. *J. Am. Chem. Soc.* **1990**, 112, 3607.
- 3.27. Gajewski, J. Weber, R. Braun, R. Manion, M. Hymen, B. *J. Am. Chem. Soc.* **1977**, 99 (3), 816.
- 3.28. Breuer, E. Bannet, D. *Tetrahedron*. **1978**, 34 (7), 997.
- 3.29. Tamao, K. Kakui, T. Kumada, M. *Tetrahedron Lett.* **1979**, 7, 619.
- 3.30. Matsui, S. *Bull. Chem. Soc. Jpn.* **1984**, 57 (2), 426.
- 3.31. Mc Greer, D. Chui, N. *Can. J. Chem.* **1968**, 46, 2225.
- 3.32. Gung, B. Yanik, M. *J. Org. Chem.* **1996**, 61, 947.
- 3.33. Williams, D. Fleming, I. *Spectroscopic Methods in Organic Chemistry*. 2nd. ed. McGraw-Hill Book Company (U.K.) Ltd., England. **1973**.
- 3.34. Silverstein, R. Bassler, G. Morrill, T. *Spectrometric Identification of Organic Compounds*. 4th. ed. John Wiley & Sons, Inc., New York. **1981**.

## Conclusion

The work presented in this thesis has described the development of conformationally restricted and epoxide-based peptidomimetics.

A dipeptide surrogate, which contains a 1,2-pyrrole moiety, has been developed as a conformationally restricted *cis* peptide bond mimic. The amino terminus of the mimic was protected with Boc, and chain extension, by coupling of an amino acid residue at the carboxyl terminus, was achieved. Future research in this area could involve the incorporation of the *cis* peptide bond mimic into a biologically active peptide.

A series of epoxide-based peptidomimetics, designed as inhibitors of the HIV PR, have been developed. A series of pseudosymmetrical epoxides were designed to capitalise on the unique symmetry of the HIV PR. Unfortunately, these compounds were found to be inactive. However, a further series of epoxide-based compounds, inspired by the known inhibitor of the HIV PR, 1,2-epoxy-3-(*p*-nitrophenoxy)propane, displayed good inhibitory activity.

Towards the synthesis of the epoxide-based peptidomimetics, an investigation of the “Horner-Wadsworth-Emmons” (HWE) reaction was undertaken. The goal being to establish the conditions conducive to the formation of the *Z* olefin. It was found that to maximise the proportion of *Z* olefin a reaction should be performed at -78 °C, in THF, with methyl diphenylphosphonoacetate. Future research in this area could involve an investigation into the affect of the cation employed in the reaction.

## Glossary of Abbreviations

Acetyl chloride	AcCl
Acquired Immune Deficiency Syndrome	AIDS
Alanine	Ala
Arginine	Arg
Asparagine	Asn
Aspartic acid	Asp
Benzotriazolyloxy-tris-(dimethylamino)phosphonium hexafluorophosphate	BOP
Benzyl	Bzl
2-( <i>tert</i> -Butoxycarbonyloxyimino)-2-phenylacetonitrile	"Boc-on"
<i>tertiary</i> -Butyl	<i>tert</i> -Bu
<i>tertiary</i> -Butyl hydroperoxide	TBHP
<i>n</i> -Butyl lithium	<i>n</i> -BuLi
Carbobenzyloxy	Cbz
Correlation spectroscopy	COSY
Cystine	Cys
Dicyclohexylcarbodiimide	DCC
Dicyclohexylurea	DCU
Diethylazodicarboxylate	DEAD
Distortionless enhancement by polarisation transfer	DEPT
Diisobutylaluminiumhydride	DIBAL
L-(+)-Diisopropyl tartrate	DIPT
1,2-Dimethoxyethane	DME
4-Dimethylaminopyridine	DMAP
1,2-Epoxy-3-( <i>p</i> -nitrophenoxy)propane	ENP
Gas chromatography mass spectrometry	GCMS
Glutamic acid	Glu
Glycine	Gly
Heteronuclear correlation	HETCOR
Heteronuclear multiple quadrupole correlation	HMQC
1,4,7,10,13,16-Hexaoxacyclooctadecane	18-crown-6
Histidine	His

“Horner-Wadsworth-Emmons”	HWE
Human Immunodeficiency Virus	HIV
Human Immunodeficiency Virus Protease	HIV PR
1-Hydroxybenzotriazole	HOBT
Isoleucine	Ile
Leucine	Leu
Lysine	Lys
Methionine	Met
Norleucine	Nle
Nuclear Overhauser enhancement	nOe
Optical rotation	$[\alpha]_D$
Oxalyl chloride	Oxalyl Cl
Phenylalanine	Phe
Potassium bis(trimethylsilyl)amide	KN(TMS) <sub>2</sub>
Potassium <i>tertiary</i> -butoxide	<i>tert</i> -BuOK
Proline	Pro
iso-Propyl	iso-Pr
Room temperature	r.t.
Ruthenium trichloride hydrate	RuCl <sub>3</sub>
Serine	Ser
Simian Immunodeficiency Virus	SIV
Statine	Sta
Tetrazole ring	$\psi[\text{CN}_4]$
Threonine	Thr
Tryptophan	Trp
Tyrosine	Tyrr
Valine	Val



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